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The REMIRA (REMission in RA) study

Defining Low Disease Activity (LDA) states using clinical, imaging and biological measures in Rheumatoid Arthritis

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PhD Thesis

The REMIRA (REMission in RA) study: Defining Low Disease Activity (LDA) states using clinical, imaging and biological measures in Rheumatoid Arthritis

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Abstract

Rheumatoid Arthritis (RA) is a heterogeneous disease with variable outcomes. Its contemporary treatment has made remission a reality with an increasing proportion of patients achieving this target. The goal of this thesis was to improve knowledge of RA remission and its clinical implications. It had three aims. Firstly to define remission and sustained remission using an extended range of clinical, laboratory and radiological biomarkers. Secondly to identify clinical and laboratory predictors of remission. Thirdly, to assess the impact of remission on health-related quality of life.

Systematically reviewing published early RA studies showed 17% achieved remission using the most stringent criteria and 33% using the least stringent criteria. Intensive treatment increased the frequency and structural benefits of remission. Modelling studies in early RA patients from an early RA trial and large observational cohort showed remission was predicted by age, gender, and tender joint count. Studies in a new, unique cohort of 104 RA patients with stable low disease activity followed for 12 months (REMIRA) showed remission was frequent with the least stringent criteria and rare with the most stringent ones. Only a minority of patients achieved sustained remissions. Ethnicity and conventional disease activity assessments predicted sustained remissions. Sustained remissions were also predicted by the multi-biomarker disease activity score and four of its components (highly sensitive C-reactive protein, serum amyloid-A protein, interleukin-6 and leptin). Finally, achieving sustained remission maximised quality

of life outcomes compared to low disease activity states throughout the study period.

This thesis has made novel contributions towards the understanding of RA remission which has important impact in the clinical setting. The use of clinical and laboratory biomarkers can predict sustained remission and therefore guide treatment decisions. With the growing emphasis on personalised medicine, this thesis brings us one step closer to achieving individualised care in RA.

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Acknowledgements

Working on this PhD has been a wonderful and unforgettable experience. It has allowed me time to develop my interest in translational research. I have gained numerous skills through this process including learning the art of musculoskeletal ultrasound, to fine-tune my clinical examination skills at detecting low-grade synovitis and in conducting a research study. I have also furthered my generic skills in giving talks, writing papers and working within a non-medical team as well as within the commercial sector. In the lab, I've learnt numerous new techniques including flow cytometry, mRNA isolation, cell culture and the art of biobanking.

I am indebted to many people:

First and foremost, I would like to express my gratitude to the invaluable help, advice, support and guidance from my supervisors, Professors David Scott and Andrew Cope. Not only have they guided me through my PhD but also through my career as a rheumatologist and allowed my interest in research to flourish. Prof Scott, my mentor, who has always given me good and solid advice on career, having a family and generally on life. Professor Cope, the forever optimist, who was always able to find that glimmer of hope in times of despair, particularly when all I could find were p values of > 0.05 ! I thank my PhD committee, the chair, Professor Gabriel Panayi as well as Profs Jonathan Barker and Giovanna Lombardi for all their objective input into the progress of my PhD. To my previous supervisor, Prof Gabrielle Kingsley, I thank her for all her support and advice over my years as a trainee. She has played an integral part to my success in obtaining the NIHR doctoral research fellowship.

To the The Cope Lab, the past and present members: Tharsana Tharmalingam, Joanna Clark, Suchitra Chinthapalli, Esperanza Perucha, Georgina Cornish, Garth Burns, Manoj Saini, Cristina Sanchez-Blanco, Janet Giles, Amanda Boerneke, thank you for making the lab such a happy and enjoyable experience. I am grateful to all their support and advice. I feel very fortunate to have been be surrounded by so much expertise and humbled by their dedication to furthering science. During my PhD, I had the pleasure of supervising 2 bright and enthusiastic students: Chanaka Dahanayake, an intercalated BSc student, who was a great help with the RF and ACPA assays and Suchitra Chinthapalli, an academic FY2, who helped tirelessly with the biobanking of the precious REMIRA samples. I am also grateful to the rest of the CMCBI lab: Geissman, Taams and Corrigall lab for their willingness to help and give advice. Dr Matthew Arno, the Genomics Centre manager and the rest of the genomics team, who donated their time generously to help with isolation of mRNA and preparation of the samples to be sent for analysis.

Ultrasound played a significant role in my REMIRA dataset. I thank Professor Peter Taylor and Dr Stephen Kelly for their advice on the set-up of the ultrasound aspect of the study. I am also grateful to Dr Toby Garrood for all his time and dedication to scanning all the REMIRA patients at baseline and T12 as well acting as my second reader.

To Fowzia Ibrahim, the departmental statistician and friend, I am forever grateful for her patience with my endless emails and queries on STATA and the analysis of my datasets. In order for the remission systematic review to be published, I am

also grateful for the help of Dr Ian Scott who acted as the 2nd researcher to run the search strategy.

To the present and past members of the Crescendo Bioscience team, Guy Cavet, Soraja Ramanujan, Doug Haney, Nadine Defranoux and WanYing Li for all their contributions to the numerous abstracts that we have submitted to the BSR, EULAR and ACR. I have really enjoyed our collaboration together and I particularly enjoyed my mini-sabatical to San Francisco. I would like to thank them for being such great hosts.

I am very grateful to the research nurses at all 3 sites for their help: Christina Blanco-Gil, Aderonke Audu and Debbie Johnson as well as all clinical staff for their help in patient recruitment. I am also hugely indebted to the patients who kindly their time and blood (!) to this study. Without them, this REMIRA study would not exist.

I gratefully acknowledge the National Institute of Health and Research for funding my work through the doctoral research fellowship (NIHR/DRF/2009/02/086).

Last but not least, I thank my family: my parents and my parents-in-laws for their understanding and continuing support, my loving husband for being my rock and my little son who can always brighten up my day.

*I dedicate this thesis to my parents, my parents-in-laws, my husband and my son
for all their unconditional love and whole-hearted support. Thanks for putting up
with me in the past few painful months! With all my love....*

Abbreviations

95% CI	95% Confidence Interval
ACR	American College of Rheumatology
ACPA	Anti-citrullinated peptide antibody
Anti-TNF	Anti-Tumour Necrosis Factor
ARA	American Rheumatism Association
AUC	Area under the curve
BeST study	Behandel-Strategieën (treatment strategies) study
BRASS	Brigham and women's hospital RA sequential study registry
CAMERA study	Computer assisted management of early RA
CARDERA study	Combination anti-rheumatic drugs in early RA
CONSORT	Consolidated standards of reporting trials
CRP	C-Reactive Protein
CsA	Ciclosporin A
CXCL10	C-X-C motif chemokine 10
DAS	Disease Activity Score
DGH	District General Hospital
DMARDs	Disease Modifying Anti-Rheumatic Drugs
ERAN	Early Rheumatoid Arthritis Network
ESR	Erythrocyte Sedimentation Rate
EMS	Early Morning Stiffness
EULAR	European League Against Rheumatism
EGF	Epidermal growth factor

FACIT – F	Functional assessment of chronic illness therapy – Fatigue
FDA	Food and Drugs Administration
FINRACO study	Finnish RA Combination Therapy
HACA	Human anti-chimeric antibody
HBSS	Hank's Balance Salt Solution
HRQoL	Health related quality of life
hsCRP	High sensitive C-Reactive Protein
IA	Intra-articular Injection
ICC	Intraclass Correlation Coefficient
IL 6	Interleukin 6
IM	Intramuscular Injection
InFoRM	The index of RA measurement study
IQR	Inter-quartile range
JIA	Juvenile Idiopathic Arthritis
KCH	King's College Hospital
LDAS	Low disease activity state
MBDA score	Multi-biomarker disease activity score
MCID	Minimum clinically important changes
MCP1-5	Metacarpophalangeal joint 1-5
MMP1	Matrix metalloproteinase 1
MMP3	Matrix metalloproteinase 3
MRI	Magnetic resonance imaging
MTP	Metatarsophalangeal joint
MTX	Methotrexate

NICE	National Institute of Health and Clinical Excellence
NTT	Number needed to treat
PBS	Phosphate buffered saline
PIP	Proximal interphalangeal
RA	Rheumatoid Arthritis
RCT	Randomised Control Trial
REMIRA study	Remission in Rheumatoid Arthritis study
RF IgA	Rheumatoid Factor IgA isotype
RF IgG	Rheumatoid Factor IgG isotype
RF IgM	Rheumatoid Factor IgM isotype
SAA	Serum amyloid A
SD	Standard Deviation
SF36 MCS	Medical Outcomes Study 36-Item Short-Form Health Survey Mental component score
SF36 PCS	Medical Outcomes Study 36-Item Short-Form Health Survey physical component score
SJC	Swollen Joint Count
SSZ	Sulfasalazine
TICORA study	Tight control for rheumatoid arthritis
TJC	Tender Joint Count
TNF-R1	Tumour necrosis factor receptor 1
OR	Odds Ratio
UHL	University Hospital Lewisham
VAS	Visual Analogue Score

VCAM1	Vascular cell adhesion molecule 1
VEGF-A	Vascular endothelial growth factor – A
vdH-SS	van der Heijde modification of the Sharp Score
YKL-40	Human cartilage glycoprotein-39

1 Introduction

1.1 Rheumatoid Arthritis

Rheumatoid Arthritis (RA) is a chronic, multi-systemic, inflammatory disease characterised by uncontrolled proliferation of the synovial tissue. This leads to damage of the joints and ultimately loss of function and disability. It is the most common cause of chronic inflammatory joint disease, occurring in 0.8% of the adult population in the UK and is more common in women. In addition to joint damage, RA can also cause extra-articular manifestations including vasculitis, anaemia, osteoporosis, pulmonary involvement and psychological disorders. It is also associated with an increased risk of cardiovascular disease.

The economic burden of RA is considerable for patients as well as for the health care and social services (Pugner et al. 2000, Kobelt and Jonsson 2008). About 30% of patients are unable to work within 10 years of onset of RA and 50% after 20 years (Scott et al. 1987, Wolfe, Hawley 1998). The total costs of RA in the UK, including indirect costs and work related disability have been estimated at between £3.8 - 4.75 billion per year (NICE 2009).

In the last 10 years, there have been major advances in the treatment of RA. Studies have shown that early and aggressive treatment is important to improve clinical outcomes. In addition, when compared to DMARD monotherapy, therapeutic regimes using combination DMARD therapy or anti-TNF therapy combined with Methotrexate have been shown to be superior in terms of clinical and radiographic outcomes in early RA (Breedveld et al. 2006, Breedveld et al.

2004, Smolen et al. 2009a, Keystone et al. 2009) and established RA (Klareskog et al. 2004, Maini et al. 1998). The aim of RA therapy has now shifted from symptomatic control to strategies aiming at inducing and maintaining remission.

1.2 Clinical presentations of RA

RA is the most common chronic inflammatory joint disease. There are no single clinical sign, symptom, test nor histological finding which is pathognomonic of rheumatoid arthritis. It is a heterogeneous disease diagnosed by applying a combination of clinical findings and laboratory tests. It has a highly variable disease onset and disease course. The presentation can vary from a gradual, insidious onset to explosive acute disease onset with fever, polyarthritis and extra-articular features. Articular signs and symptoms include pain, swelling and stiffness in a symmetrical manner involving small joints of the hands and feet. The typical clinical finding of synovitis includes soft tissue swelling and tenderness (Figure 1.1). Concomitant bursitis, tenosynovitis and carpal tunnel syndrome may be present. Systemic symptoms include generalized weakness, weight loss and low-grade fever.

If patients are left untreated or under-treated, joint deformities can occur. These include Boutonniere's deformity, swan neck, joint subluxation, ulnar deviation and tendon rupture (Figure 1.1). Atlantoaxial subluxation of the cervical spine is rare but potentially life-threatening complication of RA.

Figure 1-1: Clinical presentation of Rheumatoid Arthritis.

A) Early RA. Symmetrical swelling of proximal interphalangeal joints, the metacarpophalangeal joint B) Established RA with chronic changes: ulnar deviation, Z-thumb and MPCJ subluxations. Picture courtesy of ACR image bank



(A) Early RA



(B) Established RA

The incidence and prevalence of RA is 2-4 times higher in females than men. This ratio decreases with age. It can affect patients of any age but its incidence rate seems to increase with age up to a plateau of around 60 years. Elderly-onset RA is classified as patients with a diagnosis of RA after the age of 65.

One variant of RA is palindromic rheumatism which presents with episodes of acute polyarthritis involving one or more large or peripheral joints. These episodes last from hours to days and there is spontaneous resolution of symptoms and signs in between attacks. Approximately one third of patients will evolve into classical RA.

The laboratory tests include elevated ESR and CRP. Thrombocytosis, leucocytosis and anaemia are common. RF (rheumatoid factor) and ACPA (anti-citrullinated peptide antibody) are important serological tests are important for diagnosis and for prognosis. These serological biomarkers will be discussed in more details in

sections 1.5 and 1.6. Imaging is useful for the diagnosis of rheumatoid arthritis. Conventional radiographic examinations are still the gold standard for assessing joint damage. The use of ultrasound has become standard practice in clinics. It is of use at disease onset to detect subclinical synovitis of symptomatic joints. In addition, it also has a role in assessing erosions which may not be detectable on conventional x-rays. MRI has also been shown to be sensitive at detecting early synovitis. However, due to its costs and availability, its use in clinical practice is limited. These imaging modalities are discussed in more detail in sections 1.10.

Extra-articular manifestations and RA-related comorbidities include (EULAR textbook):

1. Secondary osteoporosis
2. Muscle weakness may be related to neuropathy, steroid use or joint involvement
3. Secondary Sjogren's
4. Eye complications including Scleritis or episcleritis
5. Increased risk of malignancy including lymphomas
6. Vasculitis
7. Pulmonary involvement including pleuritis, interstitial lung disease, or nodules
8. Pericarditis
9. Increased risk of coronary heart disease
10. Secondary amyloidosis

1.3 Classification of RA

The classification for RA used in this thesis is the American Rheumatism Association (ARA) 1987 revised criteria (Arnett et al. 1988). They require four of the following:

1. Early Morning Stiffness ≥ 1 hour before maximal improvement for >6 weeks
2. Soft tissue swelling of ≥ 3 joint areas ≥ 6 weeks
3. Symmetric arthritis ≥ 6 weeks
4. Hand joint involvement (PIP/MCP/wrists)
5. Subcutaneous nodules
6. Positive test for rheumatoid factor
7. Radiographic erosions or pericarticular osteopenia in hand or wrist

After this thesis has started, the new 2010 ACR /EULAR criteria for RA were released (Aletaha et al. 2010). They require $\geq 6/10$ points for a definitive classification of rheumatoid arthritis. The new criteria were not used in this thesis as patient recruitment preceded their publication.

Table 1-1 New 2010 ACR/EULAR criteria for RA (Aletaha et al. 2010)

Patients who have at least 1 joint with definite clinical synovitis (swelling) with the synovitis not better explained by another disease*

<i>A. Joint involvement</i>	<i>Score</i>
1 large joint	0
2-10 large joints	1
1-3 small joints (with or without involvement of large joints)	2
4-10 small joints (with or without involvement of large joints)	3
>10 joints (at least 1 small joint)	5
<i>B. Serology (at least 1 test result is needed for classification)</i>	
Negative RF and negative ACPA	0
Low-positive RF or low-positive ACPA	2
High-positive RF or high-positive ACPA	3
<i>C. Acute-phase reactants (at least 1 test result is needed)</i>	
Normal CRP and normal ESR	0
Abnormal CRP or abnormal ESR	1
<i>D. Duration of symptoms</i>	
<6 weeks	0
≥6 weeks	1

Patients have to score 6 or more to fulfil criteria for RA

*The criteria are aimed at classification of newly presenting patients. In addition, patients with erosive disease typical of rheumatoid arthritis (RA) with a history compatible with prior fulfillment of the 2010 criteria should be classified as having RA. Patients with longstanding disease, including those whose disease is inactive (with or without treatment) who, based on retrospectively available data, have previously fulfilled the 2010 criteria should be classified as having RA.

1.4 Pathogenesis of Rheumatoid Arthritis

There is marked heterogeneity in RA in terms of clinical presentation, natural history, outcomes and responsiveness to treatment. The pathogenesis of RA is far from understood. On a background of genetic susceptibility, an external trigger (eg cigarette smoking, infection or trauma) triggers an autoimmune reaction leading to synovial hypertrophy and chronic joint inflammation.

It is thought that triggers of the innate immune system activate peripheral dendritic cells. These antigen presenting cells then migrate to lymph nodes, where

they present the antigen to T-lymphocytes, forming a complex of antigen, class II major histocompatibility complex and T-cell receptor. Other co-stimulatory molecules such as cytotoxic T-lymphocyte-associated antigen-4 and CD28 are also involved in the interaction. B cells are also activated which then generates antibodies RF and ACPA. This process defines the activation of the adaptive immune system. Effector T lymphocytes (Th1 and Th17) then proliferate and migrate into the joints. Cell migration is enabled through increased expression of adhesion molecules and chemokines within the synovial microvessels. This process of neoangiogenesis is induced by local hypoxic conditions and cytokines. These changes within the synovium combined with synovial architectural reorganization and local fibroblast activation, permit the buildup of synovial inflammatory tissue in rheumatoid arthritis (Scott, Kingsley 2006, McInnes, Schett 2011, Figure 1.2).

Pro-inflammatory cytokines of importance in RA include TNF- α , IL-1, IL-6 and IL-17. This immune response is then amplified by stimulating mononuclear cells, synovial fibroblasts, chondrocytes and osteoclasts. Erosion of cartilage and bone is associated with the formation of a proliferating pannus. The interface between pannus and cartilage is occupied predominantly by activated macrophages and synovial fibroblasts that express matrix metalloproteinases (MMPs) and cathepsins, which are cartilage-degrading enzymes (Scott, Kingsley 2006, McInnes, Schett 2011).

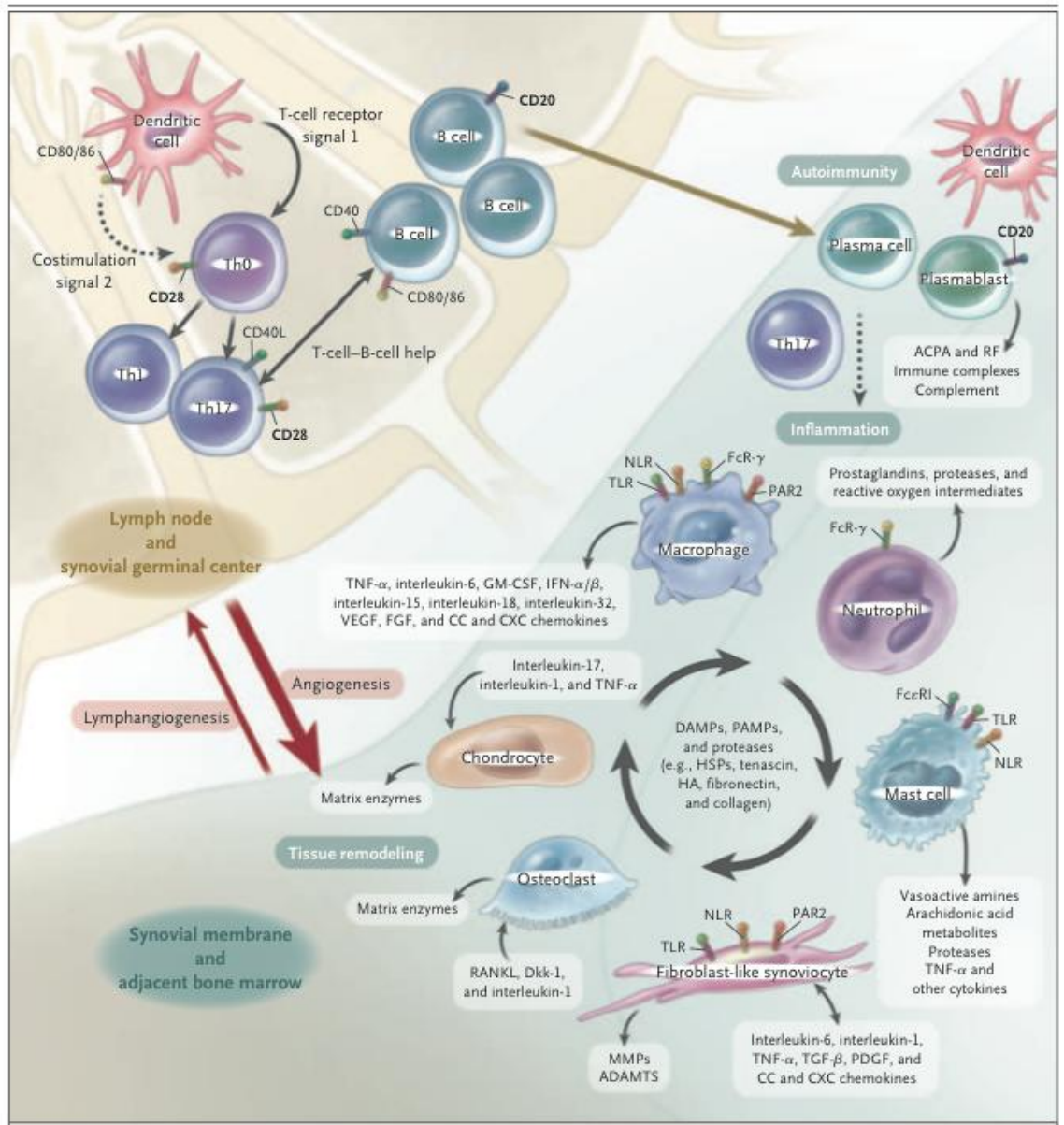


Figure 1-2 Pathogenesis of Rheumatoid Arthritis
Innate and adaptive immune processes within the joint in Rheumatoid arthritis
 (McInnes, Schett 2011)

1.5 Rheumatoid Factor

Rheumatoid Factor (RF) was the first serological biomarker to be identified in RA. It was first observed by Waaler in 1940 when a factor in sera of patients with RA agglutinated red blood cells sensitized with immunoglobulin IgG antibodies (Waaler 2007). It binds to the $\gamma 2$ - $\gamma 3$ cleft on the Fc portion of IgG. Though originally observed as an IgM antibody which agglutinated latex particles coated with human IgG, RF is known to also include both IgA and IgG isotypes. IgM isotype remains the most commonly analysed diagnostically. Numerous detection methods exist to detect RF, including latex and agglutination tests, as well as more quantitative ELISAs. It is present in 70-90% of patients with RA. RF remains an important criterion in the ARA criteria for the classification of Rheumatoid arthritis. With the newer ACR 2010 revised criteria for classifying RA (Aletaha et al. 2010), levels of RF titres (negative/low-positive/high-positive) are incorporated within this revised criterion. RF is detectable in a number of other autoimmune conditions, such as Sjögren's syndrome (60-80%) and Cryoglobulinaemia (40-100%) as well as infectious diseases. Furthermore, RF has been found in healthy individuals, particularly with older age (5%). Nevertheless RF detection is still of clinical importance in RA. High titres have been associated with more a severe disease state, whilst the presence of both IgM and IgA isotypes has been demonstrated to have a high diagnostic value (Jonsson et al. 1998), and the presence of all three isotypes produced a positive predictive value of 96% (Swedler et al. 1997). Studies have suggested that IgA RF positivity is associated with more active RA, increased joint damage and a higher frequency of extra-articular manifestations (Jonsson, Valdimarsson 1998). IgA RF positivity in healthy

people is also a predictor of the development of RA (Rantapaa-Dahlqvist et al. 2003). IgG RF is often present in sera and synovial fluid of patients with severe RA. Its role in monitoring response to therapy has been described in rheumatoid vasculitis (Scott et al. 1981).

1.6 Anti-cyclic citrullinated peptide antibodies

Antibodies to perinuclear factor (Nienhuis, Mandema 1964) and keratin (Young et al. 1979) have been previously shown to be highly specific for RA. These antibodies are directed against citrulline containing epitopes. Citrulline is not a naturally occurring amino acid as it is not incorporated into proteins during protein synthesis. It is generated via post-translational modification of arginine residues by one of a family of enzymes called peptidylarginine deiminases (PAD). Subsequent studies have shown autoantibodies reactive to cyclic synthetic peptides containing citrulline detected using an ELISA based assay were highly specific to RA. This formed the basis of the anti-CCP test. In an effort to improve the sensitivity of this test, a dedicated library of synthetic citrulline-containing peptides were screened with RA sera and a new set of peptides (CCP2) was developed giving superior performance compared to the CCP1 test. Anti-CCP detection in the routine clinical laboratories is principally derived from the anti-CCP2 assay, formed from a large scale screening of unknown citrullinated peptides using RA sera (Vossenaar, van Venrooij 2004).

Anti-CCP antibodies are also termed anti-citrullinated peptide antibodies (ACPAs). It is present in approximately 70-80% of RA patients. The revised ACR/EULAR

2010 RA classification criteria has included the use of ACPA. A recent systematic literature review found the assay had specificity for RA, ranging from 81-100% and sensitivity ranging from 39-94% (Avouac, Gossec & Dougados 2006). ACPA can be present early in disease and may precede onset of symptoms by many years (Rantapää-Dahlqvist et al. 2003). It can also predict the development of RA in patients with early undifferentiated arthritis (van Gaalen et al. 2004). ACPA has been reported as a better predictor of more severe disease than RF (Kastbom et al. 2004) and the presence of ACPA is a predictor of radiographic damage at baseline and progression over 24 months (Vencovsky et al. 2003).

1.7 Assessment of Disease Activity in Rheumatoid Arthritis

The symptoms and signs of RA vary over time and between individual patients. Clinical variables range from early morning stiffness, joint pain, joint swelling and functional impairment to more general symptoms such as fatigue and impairment of general health. Because of this variety in disease expression, a selection of variables is needed to assess disease activity. In clinical trials, disease activity is based on sets of clinical variables developed by EULAR or ACR. The EULAR response criteria use the individual change in the composite score DAS28 and the level of DAS28 reached whereas ACR response measures percentage change. Despite their differences, they were found to be in reasonable agreement in the same set of clinical trials (van Gestel et al. 1999).

The advantages of DAS, DAS28 and EULAR response criteria include:

- 1) DAS and DAS28 are continuous scales and reflect the degree of underlying inflammation whereas ACR response criteria are a measure of change.
- 2) DAS has a Gaussian distribution and is easier to interpret in clinical trials.
- 3) DAS is also sensitive enough to assess small effects.
- 4) As it is an absolute number, responses to treatment in trials can be compared.
- 5) Trial results can be expressed as a clinically meaningful outcome, which can be translated into the clinical setting.

1.7.1 Disease Activity Score (DAS)

The disease activity score (DAS) was developed on the basis of a large prospective study in which the decision of rheumatologists to start a DMARD because of disease activity or to stop treatment because of disease remission were equated with high and low disease activity, respectively (van der Heijde et al. 1990, van der Heijde et al. 1993). Data from early RA patients (< 3 yrs) were used. Following this, a new DAS formula was developed using the same procedure and same cohort but with up to 9 years of follow-up (Prevoo et al. 1995). The resulting DAS was almost identical demonstrating that disease duration did not influence the construction of the DAS. The original DAS consists of the following composite variables: Ritchie articular index (RAI 0-78), 44 Swollen Joint Count (SJC, 0-44), Erythrocyte Sedimentation Rate (ESR) and general health assessment on a visual analogue scale (VAS, range 0-100), where the RAI is the sum of the grades of tenderness (0 = not tender, 1 = tender, 2 = tender and causes wince, and 3 = tender, causes wince and effort to withdraw) elicited by applying firm pressure over the joint margin of

articular joints. The joints involved include sternoclavicular, acromioclavicular, shoulder, elbow, wrist, MCP, PIP, knee, ankle, and MTP

Its formula is as follows:

$$0.53938*\sqrt{\text{Ritchie Articular Index}} + 0.06465*(\text{SJC}) + 0.330*\ln(\text{ESR}) + 0.00722*(\text{General Health})$$

The DAS uses the square-root and natural log transformation to provide a Gaussian distribution and is a continuous scale ranging from 0-10. As it also contains a patient's global assessment of disease activity (VAS), it reflects patient-assessed disease activity as well. The level of disease activity is considered low (DAS <2.4), moderate (2.4-3.7) or high (>3.7). A DAS <1.6 corresponds to a state of remission according to the American Rheumatism Association (ARA) criteria.

1.7.2 DAS28

For reasons of convenience, a reduced DAS was created using fewer joints (Prevoo et al. 1995). It consists of 28 non-graded (compared to the RAI which is graded in terms of tenderness) Tender Joint Count (TJC, 0-28), 28 Swollen Joint Count (SJC, 0-28), ESR and VAS (range 0-100). The DAS28 has a continuous scale ranging from 0-9.4 and shows a Gaussian distribution in the RA populations. The 28 joints include all MCPs, PIPJs, wrists, elbows, shoulders and knees.

Its formula is:

$$0.56*\sqrt{\text{TJC28}} + 0.28*\sqrt{\text{SJC28}} + 0.70*\ln\text{ESR} + 0.014*\text{General Health}$$

DAS and DAS28 values do not directly compare but the correlation was calculated to be 0.97. The level of disease activity can be interpreted as low (<3.2), moderate (3.2-5.1) or high (>5.1). A DAS28 <2.6 corresponds to being in remission according to the ARA criteria. This means that nearly all RA patients in remission have a DAS28<2.6 but not all patients with DAS28<2.6 are in remission. The DAS28 was validated using the data from the same cohort and data from a very similar cohort. Patients' impressions of disease activity have been shown to correlate well with the DAS28 (Leeb et al. 2005). Furthermore, there is evidence from ultrasound studies that synovitis may be present in tender joints not considered to be swollen clinically (Scheel et al. 2005).

1.7.3 DAS28-CRP

An alternative formula for DAS28 has been developed which incorporates CRP instead of ESR. CRP correlates with disease activity, radiologic progression and treatment response (Nakamura 2000) and may be more preferable to ESR. DAS28 CRP has been validated in cohorts of early (Hensor et al. 2010) and established (Wells et al. 2009) rheumatoid arthritis cohorts. The correlation coefficient between DAS28-ESR and DAS28-CRP was very strong (0.95). However, DAS28CRP threshold values were found to be lower at <2.3, 2.3-2.7 and > 4.1 corresponding to remission, LDA and high disease activity respectively (Inoue et al. 2007).

Its formula is:

$$0.56*\text{SqrtTJC28}+0.28*\text{sqrtSJC28}+0.36*\ln(\text{CRP}+1)+0.96$$

$$+0.014*\text{General Health}$$

1.7.4 SDAI and CDAI

The simplified disease activity index (SDAI) is a validated and sensitive assessment of disease activity and treatment response which is comparable with the DAS28 and ACR response criteria (Smolen et al. 2003). It is an unweighted and untransformed index and therefore easy to calculate. It uses 5 variables: TJC28, SJC28, Patient VAS (PVAS; 10cm), physician VAS (PhVAS; 10cm) and CRP (mg/dl). It correlates highly with DAS28, physical function and progression of joint damage. The cut-offs for SDAI are ≤ 3.3 , 3.3-11 and > 26 corresponding to remission, LDA and high disease activity respectively. The SDAI can be further simplified in the form of the clinical disease activity index (CDAI) (Aletaha et al. 2005a) where the CRP is omitted. This also correlates well with HAQ and radiographic progression just as well as DAS28 and SDAI. The advantage of the CDAI is that assessments of disease activity in clinic can be carried out without the need for awaiting laboratory results. The cut-offs for CDAI are ≤ 2.8 , 2.8-10 and > 22 corresponding to remission, low disease activity (LDA) and high disease activity respectively.

Their formulae are:

$$SDAI = SJC28 + TJC\ 28 + PVAS + PhVAS + CRP$$

$$CDAI = SJC28 + TJC\ 28 + PVAS + PhVAS$$

1.7.5 ACR response

In 1995, the ACR criteria (Felson et al. 1993) were developed as definitions of improvement aimed for use in clinical trials. Improvement is denoted as ACR 20, ACR 50 or ACR 70 reflecting an improvement to the 20%, 50%, or 70% level in

certain parameters. These indices are now commonly used in clinical trials and this uniformity enables trials to be compared.

ACR 20:

- 20% reduction in number of tender joints and swollen joints
- Plus an improvement of at least 20% in at least 3 of:
 1. Patient's assessment of pain
 2. Patient's assessment of physical function
 3. CRP or ESR
 4. Patient global assessment of disease
 5. Physician global assessment of disease

ACR 50:

- 50% reduction in number of tender joints and swollen joints
- Plus an improvement of at least 50% in at least 3 of:
 1. Patient's assessment of pain
 2. Patient's assessment of physical function
 3. CRP or ESR
 4. Patient global assessment
 5. Physician global assessment of disease

ACR 70:

- 70% reduction in number of tender joints and swollen joints
- Plus an improvement of at least 70% in at least 3 of:
 1. Patient's assessment of pain
 2. Patient's assessment of physical function
 3. CRP or ESR

4. Patient global assessment
5. Physician global assessment of disease

1.7.6 Patient withdrawals for lack of efficacy and toxicity

Despite an aim for uniformity in clinical outcome measures, there are still wide variations which are reported in different clinical trials. Choy et al., 2005 (Choy et al. 2005) investigated the use of patient withdrawals to assess lack of efficacy and adverse events in clinical trials. These outcome measures are routinely reported in clinical trials in accordance with CONSORT (Consolidated standards of reporting trials) guidelines (Begg et al. 1996). They found similar effects with these outcomes compared to ACR 20 and 70, therefore, confirming their validity.

1.7.7 Multi-biomarker disease activity (MBDA) test for RA

Clinical tools of disease activity have a critical role in guiding treatment decisions, but they are not without flaws. The clinical components are subject to intra and inter assessor variability (Uhlig, Kvien & Pincus 2009, Marhadour et al. 2010) and can be confounded by co-morbidities such as fibromyalgia (Leeb et al. 2004) and joint damage. The biomarkers within these clinical disease activity measures are non-specific and can be elevated in a number of conditions eg age, anaemia, infection and malignancy. It can also be normal in patients with active disease (Keenan, Swearingen & Yazici 2008, Sokka, Pincus 2009).

A multi-biomarker disease activity test for rheumatoid arthritis has been developed to quantitatively and objectively characterize RA disease activity (Curtis et al. 2012, Centola et al. 2013). Multiple serum biomarkers have been reported to have a role in assessing disease activity. A multiple-stage approach was taken to identify

suitable biomarkers. DAS28CRP was chosen as the gold-standard rather than DAS28-ESR as CRP measurements can be standardized in archived samples from multiple centres. Out of 396 candidate biomarkers from the literature, 130 were considered to have adequate measurability. From these, 4 feasibility studies were carried out to prioritise the biomarkers. These yielded a set of 25 biomarkers. These were entered into the development phase. To ensure that the assays were suitable for use as a clinical diagnostic test, the assays were optimized to function in a multiplex environment with precision across time, instruments, operators and reagent lots. 703 patients in the The index of RA measurement study (InFORM) cohort were used for algorithm training. The final MBDA algorithm uses 12 biomarkers to generate an MBDA score between 1 to 100. This has been shown to have the criterion and discriminant validity as an objective measure of RA disease activity and has been validated in several large RA cohorts (the index of RA measurement observational (InFoRM) study, Brigham and women's hospital RA sequential study (BRASS) registry and the Leiden early arthritis clinic cohort. This score appears to be independent of co-morbidities.

The final algorithm consisted of 12 serum biomarkers (Figure 1.3 and 1.4):

1. YKL-40 (human cartilage glycoprotein-39)
2. Interleukin 6 (IL 6)
3. Serum amyloid A (SAA)
4. Epidermal growth factor (EGF)
5. Tumour necrosis factor receptor 1 (TNF-R1)
6. Vascular endothelial growth factor – A (VEGF-A)
7. Matrix metalloproteinase 1 (MMP1)
8. Matrix metalloproteinase 3 (MMP3)

9. Resistin

10. Leptin

11. High sensitivity C-Reactive Protein (hsCRP)

12. Vascular cell adhesion molecule 1 (VCAM1)

These 12 biomarkers measure different biological pathways involved in the pathogenesis of RA and can be broadly grouped into acute-phase response (SAA, hsCRP, IL6), hormones (leptin and resistin), growth factors (VEGF and EGF), adhesion molecules (VCAM1), skeletal-related proteins (YKL-40), matrix metalloproteinases (MMP1, MMP3) and cytokine related proteins (TNFR1). The formulae used to predict TJC28, SJC28 and PGA are found in the appendix (11.4). The MBDA (multi-biomarker disease activity) thresholds for disease activity categories were determined by translating the DAS28-CRP thresholds to the corresponding MBDA scores based on the linear relationship between DAS28CRP and MBDA score as follows:

- Remission ≤ 25
- Low Disease Activity Score 26 – 29
- Moderate disease activity $> 29 \text{ \& } \leq 44$
- High disease activity > 44

This assay, named Vectra-DA[®], is now commercially available (Crescendo Biosciences) and used widely in the US.

$$\text{DAS28-CRP} = 0.56\sqrt{\text{TJC}} + 0.28\sqrt{\text{SJC}} + 0.14\text{PG} + 0.36\ln(\text{CRP}+1) + 0.96$$

$$\text{MBDA Score} = (0.56\sqrt{\text{PTJC}} + 0.28\sqrt{\text{PSJC}} + 0.14\text{PPG} + 0.36\ln[\text{CRP}+1] + 0.96) * 10.53 + 1$$

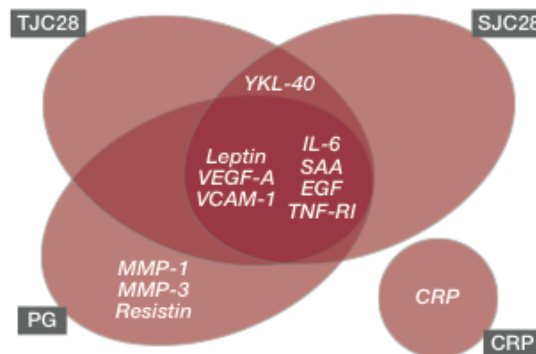


Figure 1-3: MBDA Score Algorithm.

Determination of the MBDA score from 12 serum biomarkers. The Venn diagram indicates which biomarkers are used to predict TJC, SJC and PGA scores. The resulting predictions (PTJC, PSJC, PPGA) are then combined with the CRP in the MBDA score equation shown which is analogous to that used to determine the DAS28-CRP. (Curtis et al. 2012)

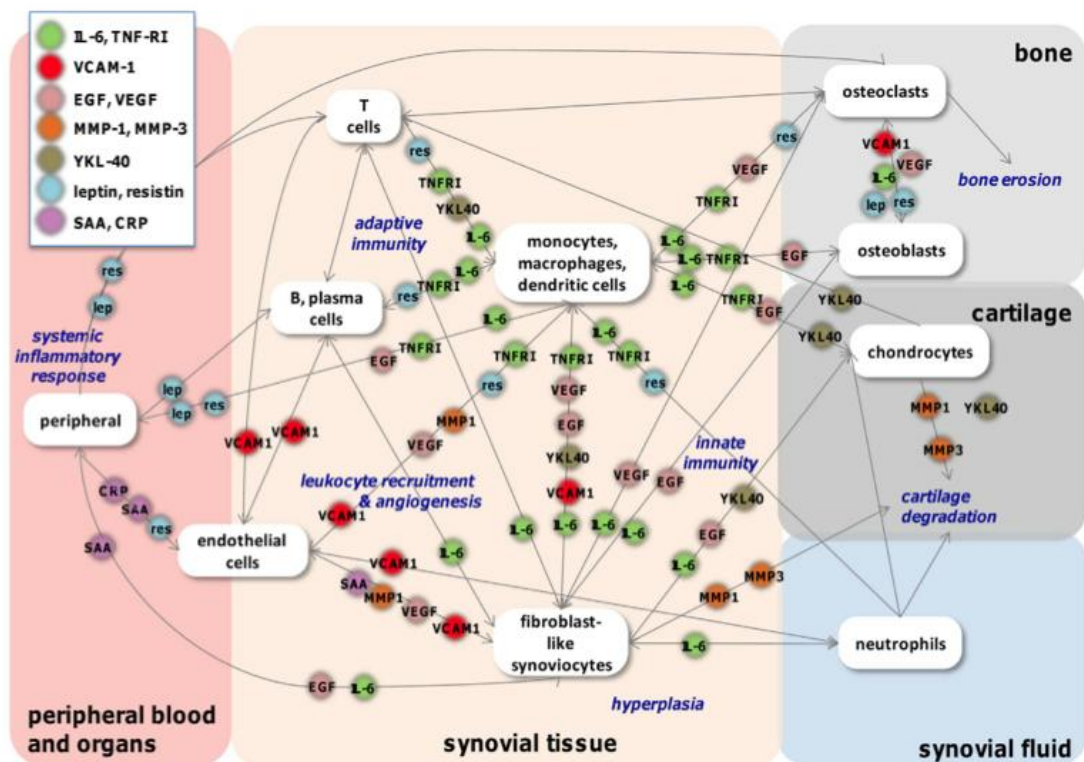


Figure 1-4 The Role of MBDA Biomarkers in RA

Network map of MBDA biomarker roles in cellular communication in RA. (Centola et al. 2013)

1.7.8 C-X-C motif chemokine 10

CXC motif chemokine 10 (CXCL10), also known as Interferon gamma-induced protein 10 (IP10) or small-inducible cytokine B10. It is a 10 kDa protein encoded by the CXCL10 gene. It belongs to the CXC subfamily chemokine containing a single and variable amino acid between the first 2 highly conserved cysteine amino acid. CXCL10 is considered to have potent inhibitors of angiogenesis properties (Belperio et al. 2000). Its expression is increased in a wide range of autoimmune diseases including Autoimmune Thyroiditis, Graves' Disease, Type 1 diabetes, systemic lupus erythematosus, systemic sclerosis, cryoglobulinaemia as well as Rheumatoid Arthritis (Antonelli et al. 2013).

It exerts its function through binding to chemokine (CXC motif) receptor 3 (CXCR3), a seven transmembrane receptor coupled to G proteins. Its secretion by CD4+, CD8+, Natural Killer (NK) and NKT cells is dependent on Interferon gamma (IFN γ). High levels of CXCL10 are therefore a marker of host immune response especially T helper 1 (Th1) cells which is of pathogenic importance in RA. CXCL10 has been detected in synovial fluid, synovial tissue and serum of RA patients (Hanaoka et al. 2003). Serum levels have also found to be high in active disease and significantly reduced with response to treatment (Kuan et al. 2010). A phase II clinical trial using an anti-CXCL10 monoclonal antibody (MDX-1100) reported increased ACR20 response rate at week 12 compared to the placebo group in active RA patients who have failed Methotrexate treatment (Yellin et al. 2012). No differences were seen in ACR50 or ACR70 responses between the treatment and placebo arms.

CXCL10 was discarded from the MBDA panel as it was only high ranking in terms of performance in 1 out of 4 feasibility studies (Curtis et al. 2012, Centola et al. 2013).

1.7.9 Calprotectin

Calprotectin, a 36.5 kDa protein, is also known as S100A8/A9, MRP-8/MRP-14, calgranulin A/calgranulin B, cystic fibrosis antigen and L1. It is a major leucocyte protein, constituting 40-60% of the soluble cytosolic protein content in neutrophilic granulocytes, as well as being a major monocyte/macrophage protein. The protein is released during cell activation and turnover. It has been named an “alarmin” which is an endogenous molecule that signals the early phase of tissue and cell damage (Andres Cerezo et al. 2011).

Calprotectin is one of the calcium binding proinflammatory S100 proteins. The protein is released during the interaction of monocytes with inflammatory activated endothelium, probably at sites of local inflammation (Frosch et al. 2000) and it binds to endothelial cells to modulate transendothelial migration of leucocytes (Vogl et al. 2004). Unlike the acute phase proteins which are mainly of hepatic origin, calprotectin is locally released at the site of inflammation. Calprotectin was excluded from the MBDA panel as the assay did not meet the performance criteria required for clinical testing.

Faecal calprotectin is routinely used for the detection of inflammatory bowel disease and is recommended by NICE for screening (NICE). In addition, high correlations between calprotectin and clinical measures have been found in

several inflammatory disease eg juvenile rheumatoid arthritis (Frosch et al. 2000), psoriatic arthritis (Kane et al. 2003), spondyloarthropathy (Kane et al. 2003), reactive arthritis (Hammer et al. 1995) and systemic lupus erythematosus (Haga et al. 1993).

In RA, calprotectin has been described within the synovial tissue (Youssef et al. 1999) and high concentrations have been found in synovial fluid and blood of RA patients (Berntzen et al. 1991). The protein has been described as a good measure of disease activity and joint inflammation in RA (Madland et al. 2002). Moreover, Calprotectin was found to be an independent predictor of x-ray damage cross-sectionally and longitudinally (Hammer et al. 2007, Hammer et al. 2010). Recently, it has been shown to correlate with ultrasound assessments which are independent of disease activity (Hammer et al. 2011).

The role of calprotectin has been explored in juvenile idiopathic arthritis (JIA) patients. Not only has it been shown to correlate strongly with disease activity (Frosch et al. 2000) but it also plays an important role in predicting good response to Methotrexate treatment (Moncrieffe et al. 2013) and predicting flares with drug tapering (Gerss et al. 2012). However, there is little in the literature describing calprotectin in low disease activity in RA. In early RA, normalisation of calprotectin levels has been reported in patients with treatment. It was found to be a predictive marker for improvement in swollen joints (Andres Cerezo et al. 2011).

1.8 Health Related quality of life assessments (HRQoL)

1.8.1 HAQ

The Health assessment questionnaire disability index (HAQ-DI) is a disease specific questionnaire for the assessment of Rheumatoid Arthritis. The questionnaire is a patient reported outcome (PRO) which is usually self-administered by the patient. (a sample questionnaire is shown in the appendix 11.15).

The following categories are assessed by the HAQ-DI:

1. Dressing And Grooming
2. Arising
3. Eating
4. Walking
5. Hygiene
6. Reach
7. Grip
8. Common Daily Activities

The patients report the amount of difficulty they have in performing some of these activities. Each question asks on a scale ranging from 0 to 3 if the categories can be performed without any difficulty (scale 0) up to cannot be done at all (scale 3). The HAQ score represents the mean of the highest values within each single domain, and therefore, is located on a scale from 0 to 3, where higher values represent worse function and vice versa. Four domains are related to dexterity (dressing, eating, reach and grip) and four to mobility (rising, walking, hygiene, and errands and chores).

The HAQ is a validated instrument for the measurement of functional status used in clinical trials (Fries et al. 1980) and is sensitive to clinically relevant changes in function. It has been shown to predict several outcomes in RA such as mortality (Pincus, Sokka 2001), work disability (Sokka, Pincus 2001) and hip replacement surgery (Wolfe, Zvillich 1998). It has also been shown to correlate with inflammation (Devlin et al. 1997). Gossec et al. (Gossec et al. 2004) followed 191 patients with early RA (disease duration less than 1 year) for 5 years and reported that patients with a baseline HAQ score <1.25 had an OR of 2.8 for the occurrence of sustained remission between the third and fifth years of monitoring. Eberhardt et al also found that a lower HAQ score was a predictor of remission for patients with disease duration less than 2 years at initial evaluation (Eberhardt, Fex 1998) .

In the literature, the HAQ score accepted as consistent with a remission state is 0.5, representing hardly any difficulties in daily activities. HAQ of 1.0 represents mild disability with some difficulties in all activities [(Molenaar, Voskuyl & Dijkmans 2002)]. However, disease duration must be considered as the reversibility of HAQ decreases with longer disease duration (Aletaha, Smolen & Ward 2006).

1.8.2 EuroQol

EuroQol also known as EQ-5D is a standardized instrument for use as a generic measure of health outcome. It is an indirect preference-based health-related quality of life (HRQoL) instrument increasingly being used for economic evaluation

of clinical interventions and health programmes. Specifically for this study, we used the EQ-5D-3L which measures health-related quality of life states consisting of 5 dimensions (mobility, self-care, usual activities, pain/discomfort, anxiety/depression) each of which can take one of 3 responses. The responses record 3 levels of severity (no problems/some or moderate problems/extreme problems) within each dimension. The combination of the five dimensions with the three answering categories produces 243 possible health states described as vectors ranging from 11111 (no problems in any domain) to 33333 (severe problems in all five domains) (e.g. 11231 corresponds to no problems walking around, no problems with self-care, some problems with performing usual activities, extreme pain or discomfort and not anxious or depressed). The EuroQol can also be reported as a preference-based, single index number. The latter is calculated by applying algorithms that link the five-digit health state description to average values for members of the general population by the time trade-off method. The EQ-5D allows for negative utility values, which theoretically correspond to health states worse than death. In addition, there is also a EQ VAS which is a 20cm vertical VAS that generates a self-rating of health-related quality of life (Anonymous1990, Brooks 1996).

1.8.3 SF36

The SF-36 (Ware, Sherbourne 1992) as patient-reported outcome measurement constitutes a questionnaire comprising 36 items. Different sets of items are organized into eight domains whose scores are obtained via summation and transformation of item values into a scale between 0 and 100, where higher values represent better health status. Domains and the respective number of comprising

items are as follows: physical function (PF; 10 items), physical role (RP; 4 items), bodily pain (BP; 2 items), general health perception (GHP; 5 items), vitality (VT; 4 items), social function (SF; 2 items), emotional role (RE; 3 items) and mental health (MH; 5 items). One single item that is not summarized is measuring the change of health status compared with the preceding year (health transition; HT). Furthermore, domains can be aggregated into two summary measures: the physical component score (PCS; including PF, RP, BP and GHP) and the mental component score (MCS; VT, SF, RE and MH). For norm-based scoring, scores of SF-36 and its summary measures are transformed to a mean of 50 (S.D.10), achieving the same mean and S.D. across the domains and summary scores. This method enables more useful and easier interpretation of all scores. The SF-36v2® Health Survey is a multi-purpose, 36-item health survey yielding a profile of two health component summary measures and eight health domain scales. It can be used across all adult patient and non-patient populations for a variety of purposes, such as screening individual patients, monitoring the results of care, comparing the relative burden of diseases, and comparing the benefits of different treatments. In the early 1990's, studies were initiated to address problems with meaning of words in some items and address well-documented shortcomings of the two role functioning scales, in version 1 of the tool. The result of the efforts was the development of the SF-36v2® Health Survey. Without increasing the number of questions, the SF-36v2® Health Survey improvements substantially increase the reliability and validity of scores and make the survey easier to understand and complete. Further, the norm-based scoring (NBS) algorithms make it possible to compare results across both versions of the SF-36v2® Health Surveys, eliminating concerns about loss of comparability. The NBS algorithms are useful to interpret

scores across the eight health domain scales of the SF-36v2®, to compare those domains with the two component summary measures.

1.8.4 FACIT-F

Fatigue is a prevalent and debilitating symptom in rheumatoid arthritis (Wolfe, Hawley & Wilson 1996). Fatigue is most often associated with pain, negative illness perceptions, sleep disturbances and low mood. It is also rated highly as an important RA outcome from the patients' perspective. It has been identified by the OMERACT group as an important core outcome measure for the assessment of RA (Kirwan et al. 2007). Its absence is used as part of certain criteria for remission (Pinals, Masi & Larsen 1981).

Many tools for assessing fatigue exist but none are specific to RA. Scales with evidence of validation for the measurement of fatigue in RA include Multidimensional assessment of fatigue (MAF), Global Fatigue index (GFI), Ordinal scores, visual analogue score, the multi-dimensional Functional Assessment of Chronic Illness Therapy Fatigue (FACIT-F), Profile of mood states (POMS) and SF36 vitality subscale (Hewlett, Hehir & Kirwan 2007). In this thesis, fatigue will be measured using a simple fatigue visual analogue score (VAS) and FACIT-F. FACIT-F is a well-validated quality-of-life instrument widely used for the assessment of chronic illnesses. Both have been validated for use in RA (Cella et al. 2005, Hewlett, Hehir & Kirwan 2007). FACIT-F (Yellen et al. 1997) is a 13-item questionnaire that assesses self-reported fatigue (range 0-52). Its use is common

amongst clinical trials (Strand et al. 2012, Cohen et al. 2006, Keystone et al. 2008a, Weinblatt et al. 2003). A fall of disease activity is associated with improvement in fatigue in these trials suggesting that disease activity and fatigue are closely linked. Although, the study by Pollard et al., 2006 has suggested that high fatigue levels are associated more with pain and depression and that the association with disease activity may be secondary (Pollard et al. 2006).

1.9 Radiographic outcome measures

1.9.1 Radiographs in Rheumatoid Arthritis

Conventional radiography remains an important part of the evaluation of patients with RA. Radiographic joint damage is one of the main outcomes in RA and is associated with functional impairment (Drossaers-Bakker et al. 1999). It occurs early in disease course. It is persistent and progressive, especially within the first 2 years of disease onset (Fuchs et al. 1989, Wolfe, Sharp 1998).

Radiographic examinations of the hands and feet are important at diagnosis as part of disease monitoring. NICE guidelines have advised that early RA patients should receive annual radiographs to monitor radiographic progression (NICE 2009). The characteristic changes seen are bone erosions and joint space narrowing (Figure 1.5). In advanced RA, the radiographs changes can also include misalignment, subluxation, dislocation, sclerosis and ankylosis. A variety of joints can be affected by RA including metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints in the hands, metatarsophalangeal (MTP) joints in the forefoot as well as joints in the mid-foot and hindfoot, the wrist, the knees, the glenohumeral joint at

the shoulder, the elbow and the cervical spine. The presence of erosions is part of the ACR/EULAR 2010 classification criteria for RA. Patients with erosive disease typical of rheumatoid arthritis (RA) with a history compatible with prior fulfillment of the 2010 criteria should be classified as having RA.

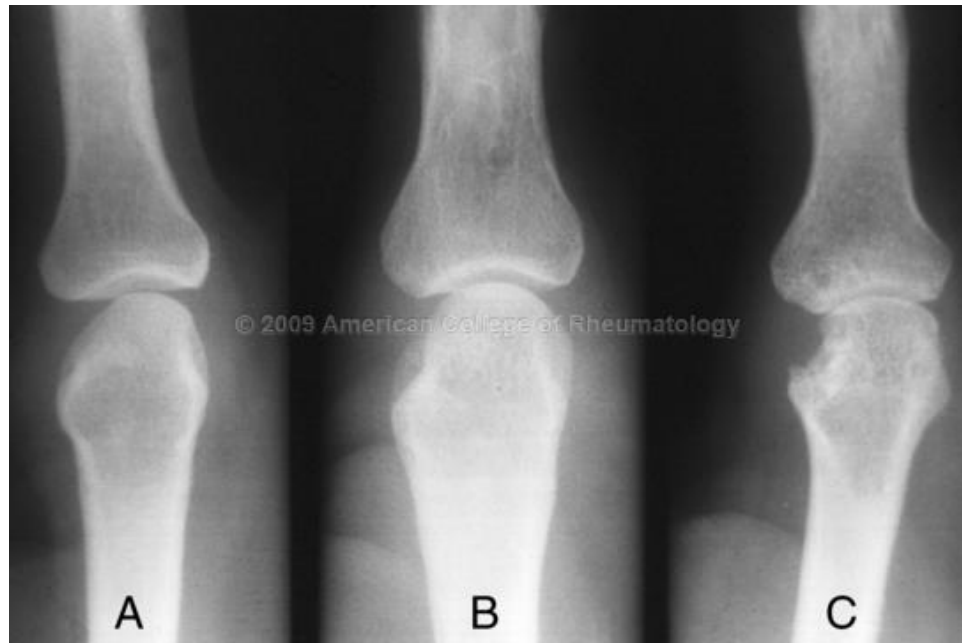


Figure 1-5: Erosion in RA.

(A) normal MCP, (B) thinning of cortex on radial side of proximal head of MCP with minimal JSN (C) marginal erosion with JSN. Picture courtesy of ACR image bank

Objective measures of damage have been developed to assess radiographic progression in clinical trials. A variety of scoring systems exists and all are based on plain radiographs of hands and feet. These include Sharp Score, van der Heijde modification of Sharp Score (vdHSS) and Larsen-Dale score.

The relationship between disease activity and radiographic progression in early RA remains a topic of debate. The follow-up study of Cohen, et al found that sustained clinical remission correlated with stability of radiological damage in most patients (Cohen et al. 2007a). However, there was radiological progression in a proportion of patients (16.7%) in sustained remission (DAS <1.6 at 3 and 5

years), and 20% developed erosions in a previously unaffected joint between the third and fifth years. Other trials have also found radiological progression in patients in remission (Molenaar et al. 2004, Brown et al. 2008). It is therefore uncertain whether radiographic progression is wholly dependent on joint inflammation (Boers 2008). Another explanation may be that current assessment tools for disease activity are insensitive at low levels of inflammation and fail to detect ongoing disease activity. Radiographic remission is not considered within the remission criteria used in clinical practice. With the newest ACR/EULAR Boolean remission criteria, radiographic remission was used as a tool for testing predictive validity of their criteria.

1.9.2 Ultrasound in Rheumatoid Arthritis

The use of ultrasound for the management of patients with early arthritis has increased over recent years in both clinical practice and research settings. The advantages of ultrasound are that it is relatively inexpensive, non-invasive and allows many joints to be assessed at any one time. It can be done in real time within the clinic and yields instant information. The main disadvantage is its dependency on the skills of the operator and potential problems with reproducibility.

Ultrasound has the capability to directly visualize both synovitis and bone damage. The 2 parameters of synovitis: Synovial Hypertrophy (SH) and Power Doppler (PD) (Figure 1.6).

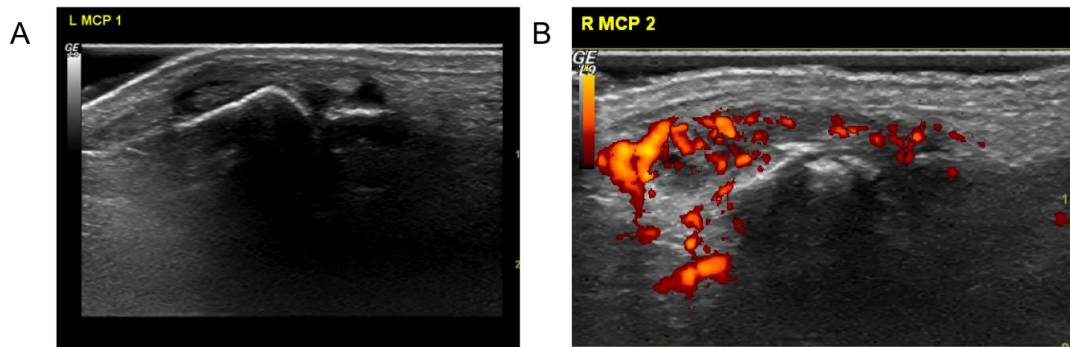


Figure 1-6: Ultrasound of Metacarpalphalangeal Joint.
(A) Synovial hypertrophy (B) Power Doppler

High resolution grey-scale ultrasonography (Fig 1.6A) allows morphological structures of the joints and surrounding tissues to be seen. Fluid and solid structures can be differentiated in their echotexture – solid structures (eg bone) are hyperechoic, whereas fluid appears anechoic. High-frequency transducers (6-14 MHz) should be used for the examination of small joints and low-frequency transducers for large joints (5-10 MHz for hip and 8-14 MHz for the knee, elbow and shoulder).

Doppler ultrasonography (Fig 1.6B) allows the blood flow to be visualized by the change in frequency of sound waves reflected by moving objects (The Doppler shift). PDUS is able to detect slow velocity flow signals eg typical for inflammation within joints and tendons.

The presence of erosions can also be assessed which is defined as a break in the cortex visualized in both longitudinal and transverse planes.

In research settings, assessments of both parameters of synovitis can be undertaken applying semi-quantitative scores or quantitative scores. The majority

of the studies use a semi-quantitative scoring system graded from 0 to 3. The number of joints evaluated can differ greatly ranging from 7 to 78 joints. In a systematic review of the reliability of ultrasound findings, good inter-observer and intraobserver reliability of still-images were found (Cheung, Dougados & Gossec 2010). However, few studies looked at the image acquisition reliability and therefore more research is still needed in this field.

Ultrasound has been shown to detect synovitis with greater precision than clinical examination in small joints of the hands and feet (Naredo et al. 2005, Filer et al. 2011). It also correlates with DAS28 and CRP (Dougados et al. 2010). Ultrasound is able to detect damage to the joints in patients with normal radiographs (Backhaus et al. 1999). It was also found to be more sensitive than MRI at detecting synovitis (Backhaus et al. 1999). There is also increasing evidence that ultrasound has a role in predicting radiographic progression. One study showed that baseline GS and US parameters were able to predict further radiographic damage at 2 years (OR 3.14 and 2.79 respectively) (Dougados et al. 2013).

1.9.2.1 Ultrasound and remission

Several studies have reported on the presence of subclinical synovitis in cohorts of remission patients. The Leeds group found that most patients classified to be in remission by their rheumatologists had evidence of synovitis on ultrasound (Brown et al. 2006), even after anti-TNF therapy (Wakefield et al. 2007). Ozgocmen et al also found a significant proportion of patients in remission (DAS28 and ACR remission) with PD signal (Ozgocmen et al. 2008).

The presence of power Doppler signal is a powerful predictor of radiographic progression (Brown et al. 2008, Foltz et al. 2012) and flare (as defined by increased treatment, Saleem et al. 2012) over 1 year. However, a major criticism of their remission cohort is that the inclusion criteria were not stringent and 23% of the patients had moderate or high disease activity at baseline. Scire et al also found the presence of PD to be a predictor of flare (within 6 months). But in addition, also found that the absence of a PD signal was associated with stable remission for 24 months. In their inception cohort of 104 patients, only 43 were in remission (Scire et al. 2009). Yoshimi et al found in their small cohort of 22 patients in remission, PD signal was associated with radiographic progression (Yoshimi et al. 2013). All these studies suggest that subclinical synovitis is ongoing in patients with clinical remission.

More stringent criteria may be more closely associated with less sub-clinical synovitis. Balsa et al., 2010, showed the superiority of SDAI over DAS28 remission criteria in the assessment of ultrasound-classified remission (Balsa et al. 2010). A more recent study using the new ACR remission criteria also demonstrated less PD signal in patients with more stringent remission criteria (Sakellariou et al. 2013). However, this was not substantiated in a study by Saleem et al., 2011 which showed similar PD signal between DAS28, SDAI and Boolean remission (Saleem et al. 2011).

1.9.3 Magnetic resonance imaging (MRI) in Rheumatoid Arthritis

MRI of joints can also be used for early diagnosis of rheumatoid arthritis and staging of disease (Figure 1.7). 3 parameters are assessed on MRI as markers in RA: Bone marrow oedema/osteitis, synovitis and erosions. Synovitis can be seen when MRI is performed with gadolinium contrast. Bone marrow oedema is a predictor of erosive progression on x-rays (Boyesen et al. 2011a, Boyesen et al. 2011b).

1.9.3.1 MRI and Remission

Studies have shown subclinical synovitis on MRI is present in patients with clinical remission and LDA states (Brown et al. 2008, Gandjbakhch et al. 2014). Gandjbakhch et al., 2014 reported that even in these clinical states, evidence of synovitis based on MRI findings are significant predictors of erosive progression (Gandjbakhch et al. 2014).

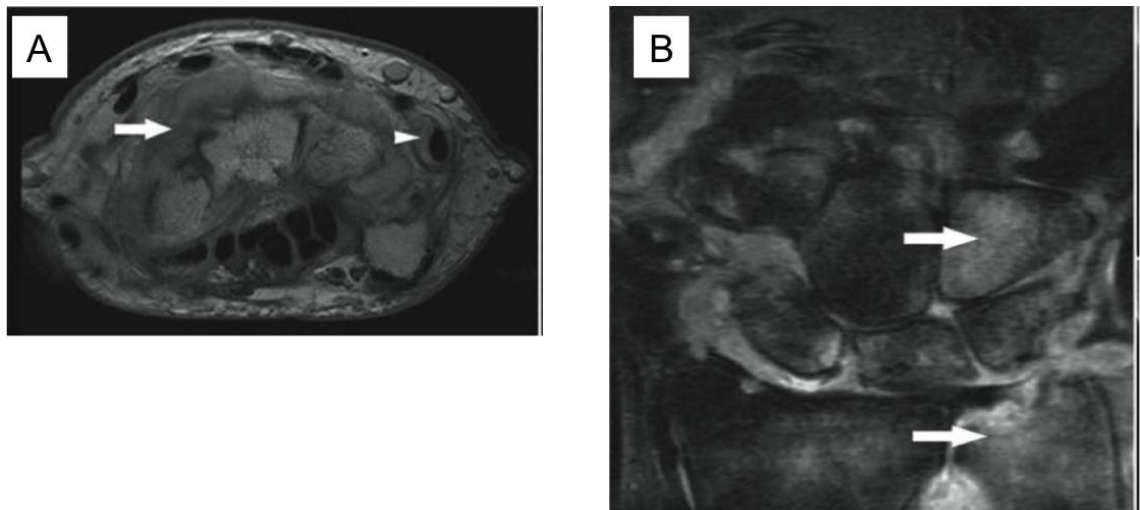


Figure 1-7 MRI of Wrist Joint

(A) T1-weighted image after gadolinium administration showing contrast enhancement of the synovium (arrow) and the sheath of the extensor carpi ulnaris tendon (arrowhead) of the wrist. (B) Coronal STIR image showing bone marrow oedema in the hamate bone and ulnar head (arrows). (Boyesen et al. 2011a)

1.10 Treatment of Rheumatoid Arthritis

1.10.1 Combination Disease Modifying Anti-Rheumatic Drugs (DMARDs)

DMARDs used in routine clinical practice include Methotrexate (MTX), Sulfasalazine (SSZ), Leflunomide, Hydroxychloroquine (HCQ) and Cyclosporin (CsA).

Methotrexate resembles folic acid and is a competitive inhibitor of folate-dependent enzymes eg dihydrofolate reductase (DHFR). These enzymes are involved in the pyrimidine synthesis and the de novo purine synthesis of DNA and RNA. The mechanisms by which MTX exerts its effects are complex. Most studies of immune function in patients with RA show only marginal effects on humoral and cellular immune responses on T and B cells, monocytes, neutrophils and fibroblasts (EULAR textbook).

Leflunomide is an isoxazole derivative and inhibits de novo pyrimidine synthesis, resulting in diverse antiproliferative and anti-inflammatory effects such as suppression of TNF-induced cellular responses and inhibition of matrix metalloproteinases and osteoclasts. (EULAR textbook).

Cyclosporin A (CsA) has complex effects on T cell function including inhibition of interleukin-2 release and subsequent activation of T cells. It is an effective DMARD but its toxicities (hypertrichosis, tremor, gum hyperplasia, hypertension and dose-related loss of renal function) has limited its use in clinical practice (EULAR textbook).

Sulfasalazine is a 5-aminosalicylic acid derivative which is metabolised via the colonic intestinal flora to sulfapyridine and 5-aminosalicylic acid (5-ASA). After absorption, it is metabolised further. Sulfapyridine is the active moiety in RA, although its mechanism of action has not been identified (EULAR textbook). It is mainly excreted in the urine, either as unchanged drug or via its metabolites.

Hydroxychloroquine, a 4-aminoquinoline derivative, is an anti-malarial agent. Its precise mechanism of action is unknown (EULAR textbook). It concentrates inside cells, within acidic cytoplasmic vesicles, resulting in changes in acidity and interference with the processing of autoantigenic peptides. It can interact with nucleic acids and inhibit endosomal toll-like receptor (TLR) activation, suggesting a potential mechanism to modulate activation of the innate immune system.

Glucocorticoids are a unique class of drugs with well-defined effects. It has pleiotropic well-characterised anti-inflammatory and immunosuppressive effects.

As the DMARD agents have different mechanisms of action, it is logical to combine these agents. Evidence for the benefits of treating RA during the early phase of the disease is accumulating (Goekoop-Ruiterman et al. 2007). Improvement in clinical and radiological outcomes has been observed. However, the optimal regimen for treating early RA has not been established. Treatment strategies are diverse, including double, triple or even quadruple combinations and may involve a step-down or step-up approach (Boers et al. 1997, Grigor et al. 2004, Mottonen et al. 1999a).

1.10.2 Biological therapies in rheumatoid arthritis

With the advent of biological therapies, treatment of RA has been revolutionised. These biological therapies consist of monoclonal antibodies and soluble receptors able to block key cytokines (TNF α , IL-1, IL-6), delete or modulate lymphocyte subsets (B cells or T cells). The biological therapies currently available include TNF inhibitors (Infliximab, Etanercept, Adalimumab, Certolizumab and Golimumab), IL-1 antagonist (Anakinra), T cell costimulation blocker CTLA-4Ig (Abatacept), B cell depleter (Rituximab) and IL-6 signal inhibitor (Tocilizumab). These treatments have significantly improved the signs and symptoms of disease, decreased the progression of joint damage, improved physical function and improved health-related quality of life (Lipsky et al. 2000, Klareskog et al. 2004, Keystone et al. 2004, Bresnihan et al. 2004, Smolen et al. 2009a, Kremer et al. 2003, Edwards et al. 2004, Genovese et al. 2008, Keystone et al. 2009, Keystone et al. 2008b).

The first part of this thesis compares the efficacy of combination DMARDs with anti-TNF with MTX therapies at inducing remission. Therefore, anti-TNF agents will be discussed in more detail in the next section.

1.10.2.1 Anti-TNF blocking agents

Amongst these biologic agents, TNF α antagonists have the largest safety data set in RA as they have been available for the last 15 years. Infliximab is a chimeric human-murine IgG1 anti-TNF α monoclonal antibody. It consists of a human immunoglobulin Fc portion and a murine TNF α binding variable region. It binds to soluble and membrane-bound TNF α and inhibits its effect by blocking TNF α and

receptor interactions. Infliximab is also cytotoxic for TNF-expressing cells (Scallon et al. 1995). It is administered as an intravenous infusion. Etanercept is a recombinant soluble p75 TNF receptor:Fc fusion protein. It consists of 2 TNF α binding domains linked to the Fc portion of human Ig. It binds to TNF α , preventing its interaction with its receptor. It also targets TNF β (lymphotoxin)(Mohler et al. 1993). It is given by weekly subcutaneous injections. Injection site reaction has been reported in 34-37% of patients compared to 7-10% of placebo (Fleischmann, Yocum 2004). Adalimumab is a fully human recombinant IgG1 monoclonal antibody. It binds to human TNF α with high affinity and therefore stops it binding to its receptors. It is given by fortnightly or weekly subcutaneous injections. Injection site reaction occurs in 19.5% of patients compared to 11.6% of placebo (Fleischmann, Yocum 2004). Both Infliximab and Adalimumab can induce neutralizing anti-globulin response that reduces their efficacy (Radstake et al. 2009). Golimumab, the newest available anti-TNF agent, requires only monthly subcutaneous injections (Keystone et al. 2009). It has also been shown to be effective in patients who have failed other anti-TNF agents (Smolen et al. 2009b).

Certolizumab pegol is a novel type of TNF inhibitor. It consists of a humanized Fab' fragment fused to a 40-kd polyethylene glycol (PEG) moiety. Certolizumab pegol binds to TNF α and prevents its interaction with specific receptors therefore neutralizing it. Its unique structure reduces immunogenicity by shielding the protein from recognition by the immune system. This leads to reduction in anti-drug antibody formation and therefore secondary failures. PEGylation also increases the half-life and therefore the drug can be administered less frequently. In animal models, PEGylation has also been shown to preferentially distribute in

inflamed tissues (Nesbitt A, et al. 2007). Certolizumab pegol does not have an Fc portion and therefore avoids potential Fc-mediated effects seen in vitro, eg complement-dependent or antibody dependent cell-mediated cytotoxicity or apoptosis (Nesbitt A et al. 2007). In addition, Certolizumab pegol is synthesized by fed-batch fermentation in *Escherichia Coli*. The benefits of this method of production include lower cost, rapid production cycle of 2-3 days, higher yield and a more reliable drug supply.

Anti-TNF agents have shown major clinical efficacy in established RA. Currently, in accordance with the NICE guidelines, anti-TNF can only be used in the UK when 2 or more DMARDS have failed. But with remission as the ultimate goal of therapy, the use of anti-TNF blockade in early RA seems a logical progression. Therefore, interest is now focused on treatment with anti-TNF agents in early RA to induce long-term impact on outcome. This will be discussed in the next section.

The use of anti-TNF blocking therapies has been limited by rare but clinically significant side effects. These include reactivation of latent mycobacterium tuberculosis, increased risk of other infections, infusion reactions, malignancies, induction of auto-antibodies, and worsening of severe heart failure. Some individuals also produce antibodies against the agents themselves. These human anti-chimeric antibodies (HACA) can neutralize the agent concerned and result in a gradual reduction of efficacy. This can lead to secondary failures.

1.10.3 Treating early disease is important

There is accumulating evidence that the course of RA is determined early. Radiographic damage is seen in more than 70% of patients with RA within the first 2 years of disease and progression is greater in the first year (Fuchs et al. 1989). Studies have shown that early and aggressive treatment significantly reduces radiographic progression and improves clinical outcomes (Nell et al. 2004, Stenger et al. 1998). In addition, a short delay in initiation of DMARD therapy has been shown to result in more rapid joint destruction and loss of function in patients with early RA (van Aken et al. 2004). Consequently the concept of a 'therapeutic window of opportunity' has been developed, which requires both early diagnosis and early intensive DMARD therapy to reduce disease progression.

Numerous trials have examined the benefit for intensive treatment in early rheumatoid arthritis. These include the use of combination DMARD therapies (Boers et al. 1997, Choy et al. 2008a, Ma, Kingsley & Scott 2010, Grigor et al. 2004) and anti-TNF therapy with Methotrexate (Emery et al. 2009, Breedveld et al. 2004, Breedveld et al. 2006, Ma, Kingsley & Scott 2010). All have shown improvement in disease activity, radiological outcomes and HRQoL outcomes over DMARD monotherapy.

1.10.4 Tight Control Treatment Regimes

Not only is aggressive treatment important in early disease to suppress disease activity, it is also important to maintain low disease activity. Welsing et al., 2004 investigated the longitudinal relationship between disease activity and radiological progression in 2 independent follow-up cohorts (Welsing et al. 2004). Fluctuating

high DAS and fluctuating low DAS showed similar rates of radiological progression to patients with a constant high DAS. Persistent low levels of rheumatoid inflammation (DAS28 <3.2) are associated with up to 50% less progression of joint damage. Disease activity (DAS) was also found to be an important factor influencing functional capacity (HAQDI) for patients with disease duration up to 6 years (Welsing, Fransen & van Riel 2005). These data support the systematic monitoring of disease activity in clinical practice to achieve persistent low disease activity. This approach is called 'tight control' regime.

The advantages of tight control regimes are:

1. There is a predefined treatment protocol to which treatments of individual patients are adjusted.
2. It is an orderly process.
3. It is useful for assessing if the treatment chosen is necessary and effective
4. It can ensure that patients are not over-treated.

Five RCTs have investigated the effects of tight control: FINRACo trial (Mottonen et al. 1999b), TICORA trial (Grigor et al. 2004), Fransen et al., 2005 (Fransen et al. 2005), BeST study (Goekoop-Ruiterman et al. 2007) and CAMERA study (Verstappen et al. 2007). 4 used early RA patients (<5 years). These studies showed that clinical and radiological outcomes are more favourable in the tight-control regime group. The range of remission rates were 37-68% vs 16-41% in the tight control group compared to normal group. This improvement in clinical and radiological outcomes did not appear to be at the cost of increased drug toxicity. Interestingly, health economic assessment of the TICORA trial showed that there

was an increased outpatient cost for the intensive strategy but this was offset by increased community healthcare and inpatient cost in the routine care group. Ultimately, the intervention was cost neutral.

BeST study used the concept of tight control in all 4 treatment groups and compared different treatment strategies. The groups using initial combination therapies with prednisolone or anti-TNF showed faster clinical improvement, however, at 2 years, there were no statistical differences between any treatment strategies. This finding was further supported by the observational trial by Verschueren et al., 2008 (Verschueren, Esselens & Westhovens 2008). They also found more remissions initially in the step up group compared to step down group but this effect had disappeared by 2 year. This showed that it was the tight-control regime principal that was important rather than the agents used for clinical outcomes. However, this is not reflected in the radiological outcomes. The initial combination groups achieved less radiological progression than the monotherapy or step-up groups. Less radiographic progression has been noted at each disease activity state in patients treated with anti-TNF therapy against conventional DMARDs (Keystone 2008). This suggests that the aim of therapy may need to be stricter for certain treatment regimes e.g DMARD monotherapy than anti-TNF therapy.

Tight control regimes are perceived as aggressive forms of treatments however, they also allow treatment reduction and hence reduce drug toxicity. CAMERA trial showed that 56% of patients in the intensive group had reduction in treatment because of sustained response. Also, the BeST trial showed that by end of 2 years,

31% of patients of step-up therapy, 36% of initial combination with prednisolone and 53% of patients on initial combination with anti-TNF had reduced their treatment to monotherapy. This will therefore minimise any potential drug toxicity of over-treatment.

The NICE guidance (NICE 2009) emphasise on treatment reduction when patients have controlled disease activity. However, currently, there is no guidance on how to reduce treatment. It is likely that more clinical, radiological or laboratory tools are required to guide treatment reductions.

1.11 Definitions of Remission

Remission has many meanings. In some medical contexts it indicates lessened disease severity. In other contexts it implies the disease has disappeared or evidence of disease activity is absent. Concepts of remission in rheumatoid arthritis (RA) reflect both models. Some definitions only indicate low disease activity states. Other definitions suggest the absence of disease, with undetectable symptoms, signs and disease markers. Critically, remission differs from “cure”, which implies RA will never return. Current opinion favours restricting remission to patients with either no or minimal synovitis, without long-term structural or functional sequelae. The seminal paper by Pinals *et al* in 1981 concluded “complete” RA remission indicates the “total absence of articular and extra-articular inflammation and immunological activities” (Pinals, Masi & Larsen 1981). However, many years later, uncertainties still remain as to how to define true biological remission states.

The introduction of new therapeutic options and strategies over the past decade has made remission an achievable goal, and in clinical practice a realistic one. An immediate consequence of this perspective is the need for an accurate and uniform way to identify remission. Although there are many definitions, remission does not yet have an internationally accepted gold standard. Remission criteria differ between studies and remission rates vary depending on the remission criteria used. Some remission criteria use categorical descriptions; the original American College of Rheumatology (ACR) remission criteria are one important example (Pinals, Masi & Larsen 1981) (Table 1-2). However, these criteria are very stringent and too few patients achieve this goal to make the definition a useful outcome to discriminate between patients in clinical trial settings, or to make it a realistic outcome in the routine clinic setting. It has been reported that the majority of healthy individuals above the age of 50 do not fulfil ACR remission criteria (Sokka et al. 2007). Consequently many variants have been described. Continuous composite measures are often used to define remission (Table 1-2); the most commonly used are the low scores calculated using the Disease Activity Score (DAS, (Prevoo et al. 1996)) or its modifications such as DAS28-ESR (Fransen, Creemers & Van Riel 2004). The newer criteria include Simplified Disease Activity Index (SDAI, (Aletaha et al. 2005b)), Clinical Disease Activity Index (CDAI, (Aletaha et al. 2005a)) and the ACR/EULAR Boolean criteria. The newest criteria, the ACR/EULAR Boolean criteria, were developed to provide a more uniform definition which can be widely used (Felson et al. 2011). It was tested for its ability to predict good functional and radiological outcomes. However, the Boolean criteria have been criticised as possibly being too stringent. In particular, the patient global visual analogue score (PtVAS) of less than 1cm (on a 10cm scale) has

proved difficult to achieve (Studenic, Smolen & Aletaha 2012). It may not even reflect disease activity as it may be more related to functional limitations, low back pain and fatigue (Masri et al. 2012).

The US Food and Drug administration (FDA) criteria for remission or complete clinical response in RA specify that patients must meet the ACR remission criteria and have radiographic arrest over a continuous 6-month period whilst not taking any antirheumatic drugs, or in the case of complete clinical response, while continuing anti-rheumatic drug therapy. This is not used routinely in clinical practice.

Table 1-2: Table of Remission Criteria

ACR = American College of Rheumatology, EULAR = European League Against Rheumatism, SDAI = Simplified Disease Activity Index, CDAI = Clinical Disease Activity Index, DAS28 = Disease activity Score 28 joints, CRP = C-Reactive Protein, ESR = Erythrocyte Sedimentation Rate, MBDA = multi-biomarker disease activity score, FDA = Food and Drugs administration

Index	Remission criteria
ACR/EULAR Boolean	TJC, SJC, CRP, PGA all ≤ 1
SDAI	≤ 3.3
CDAI	≤ 2.8
DAS28-CRP	< 2.32
DAS28-ESR	< 2.6
MBDA score	≤ 25
FDA	Old ACR remission criteria Plus no radiographic progression off treatment for 6 months
Old ACR remission criteria	5 or more over 2 consecutive months: <ul style="list-style-type: none"> • No joint swelling or soft tissue swelling of tendon sheaths • No joint tenderness or pain on motion • ESR < 30 women, < 20 men • Early Morning Stiffness < 15 minutes • Absence of joint pain by history • No Fatigue (not included in modified version)

The remission criteria that have evolved over the last two decades all reflect a similar underlying theme. Namely, changes in clinical variables assessed by clinicians such as joint counts, physician global scores and inflammatory blood markers like the erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) (Inoue et al. 2007). Despite attempts to define remission in many different forms, the definition of true remission still remains elusive. A significant proportion of patients classified in clinical remission still continue to develop radiographic progression (Proudman et al. 2007, Makinen et al. 2005a, Vazquez et al. 2007, Machold et al. 2007). This suggests ongoing sub-clinical disease in some patients apparently in remission. Radiological markers such as MRI or ultrasound may be more sensitive in detecting sub-clinical disease, though there are challenges in standardising these techniques between clinicians and centres.

Finally, there are 2 outstanding features of remission that need to be clarified. First, there is very little information about how long remission should last to be significant. Meeting criteria for remission on a single occasion is likely to be insufficient. A full clinical picture requires integrating disease states with time. Second, as more patients achieve low disease activity states, there will be more opportunities for drug tapering and withdrawal. Studies are urgently needed to determine which characteristics of remission most accurately predict those patients who are able to withdraw therapy.

In an era where treatments are increasingly targeted at a molecular level, it is important that monitoring treatment responses progresses to reflect such specific

therapies. Laboratory biomarkers, beyond ESR and CRP, are likely to play crucial roles in defining disease activity and remission in the future. It is likely that addition of radiological and laboratory biomarkers to clinical variables will help clarify the kaleidoscope-like appearance of existing remission criteria. This will give a clearer picture of what true remission entails.

1.12 Rationale of the thesis

Remission means the absence of a disease. It is conventionally assumed to mean there are undetectable or minimal symptoms and signs of the disease and that laboratory markers of active disease have returned to normal. Periods of remission can be followed by flares and subsequent periods of active disease. Due to the heterogeneous nature of RA outcomes, these periods of remission and flares are currently unpredictable.

The introduction has outlined the limitations of current knowledge about RA remission. Although several criteria have been developed to enable clinicians to judge whether or not remission is present, there is uncertainty about which criterion is the most useful as an aid for decision making in clinical practice. There is also uncertainty about the impact and clinical relevance of the length of remission. Meeting criteria for remission on a single occasion is likely to be insufficient. True remission suggests that patients are in stable remission over time. In addition, remission needs to be characterised in more detail and it is likely that addition of radiological and laboratory biomarkers to clinical variables will help clarify the concept of true remission.

1.13 Overall Aims of thesis

The overall goal of this thesis is to improve the knowledge of remission states and the clinical implications of achieving of remission in RA. The research spans three broad aims:

The first broad aim was to characterise in detail the definition of remission and sustained remission using an extended range of clinical, laboratory and radiological biomarkers.

The second broad aim was to identify predictors of remission, both for remission at single time points and also for remission sustained over periods of time. This aim reflects the disease heterogeneity of RA, the growing emphasis on personalised medicine, and the opportunity to individualise care using risk stratification to guide treatment decisions,

The third broad aim was to assess the impact of different levels of remission on aspects of RA directly relevant to patients. These include pain, fatigue and disability, which are characteristic features of established RA. Over time the disability associated with RA has considerable impacts on health-related quality of life (HRQoL) and these effects have been evaluated for both sustained and incomplete remission.

Delivering these overall aims has been achieved by focusing on five specific research objectives. These objectives have included research based on systematically reviewing the existing literature, using previously collecting clinical research data from both an observational study and a clinical trial, and establishing a large prospective cohort of RA patients in remission for this thesis.

1.14 Objectives of the thesis

The first specific objective of the thesis was to assess the frequency of remission in the published literature. A detailed systematic review was carried out to assess the

remission rates in observational and randomised controlled trials as defined by different remission criteria and to assess the impact of treatment on remission rates.

The second specific objective was to develop a predictive model for 24-month remission in early RA patients. Clinical baseline variables from a RCT comparing Methotrexate, steroids and combination DMARDs were used to develop this model (CARDERA study)(Choy et al. 2008a). The predictive model was then validated using data from a UK cohort - the Early RA Network (ERAN, (Kiely et al. 2009). Finally the clinical model was combined with serological biomarkers to develop predictors of response to different DMARD treatment regimens.

The third specific objective was to develop a cohort of RA patients with features of stable low disease activity states. This was the REMIRA cohort - REMission In RA. This cohort was used to assess the prevalence of sustained remission over 1 year and to develop predictors of sustained remission using clinical, serological and radiological biomarkers.

The fourth specific objective was to define an objective molecular signature of point remission and sustained remission and to develop molecular biomarkers in predicting sustained remission. Serum biomarkers from the REMIRA cohort will be used. These will include the MBDA score and its components as well as CXCL10 and calprotectin.

The last specific objective of the thesis was to assess the impact of remission on health-related quality of life. The impact of baseline remission and sustained remission on disability, fatigue and quality of life over 1 year were both assessed.

2 Methods and Materials

The methods and materials section is a summary of the different methods and techniques used within this body of research. Since they varied extensively, they are also summarised within each results chapter.

2.1 Systematic reviews

2.1.1 Search Terms

Pubmed, EMBASE and Medline were searched using the following search terms: Early Rheumatoid Arthritis or Early RA combined with Remission, Treatment, anti-TNF or DMARD. The search was limited to 1996 – 2008, English and Clinical trials.

2.1.2 Selection criteria

Studies were selected for inclusion using the following criteria:

1. Randomised Controlled Trials or Observational Studies
2. Patients fulfilled the 1987 ACR classification of RA
3. Disease duration less than 3 years of diagnosis.
4. Remission used as an outcome measure
5. Enrolled more than 40 patients

2.1.3 Outcomes

We included DAS (and its modifications) or ACR (and its modifications) remissions as the clinical outcome measure. Radiological outcomes of patients in remission were also assessed.

2.1.4 Quality of trials

The quality of the trials was judged using the Jadad Scoring System (Jadad et al. 1996). The Jadad scoring system, also known as The Jadad scale or the Oxford quality scoring system, is a procedure to independently assess the methodological quality of a clinical trial. It consists of 3 elements: Randomisation, blinding and withdrawals/dropouts. It ranges from 0-5, 0 being the weakest and 5 being the strongest. See appendix 11.3 for the calculations.

2.1.5 Data Extraction

Studies were assessed for eligibility and extracted data on year of publication, population source, study design, study size and follow-up period. When there were differences between observers (Margaret Ma and Ian Scott), they reviewed the papers together and came to a joint conclusion.

2.2 Autoantibody analysis

For the CARDERA and REMIRA samples, I had carried out the assays myself. RF IgM, IgA and IgG measured in relative units per ml (RU/ml) using commercially available ELISA kits (Euroimmun). Testing was performed according to the manufacturer's instructions, at a sample dilution of 1:200. The upper limit of the

normal range recommended by Euroimmun is 20 RU/ml. Anti-CCP antibodies (IgG) were measured using an ELISA based kit from Axis-Shield which detects autoantibodies towards a synthetic cyclic peptide containing modified arginine residues (CCP2 peptides). Testing was performed according to the manufacturer's instructions, at a sample dilution of 1:100. The cut-off value for anti-CCP antibody positivity was 5 U/mL.

2.3 REMIRA cohort

This is a unique cohort which I had collected. This included applying for Ethics, R+D, setting up Remission clinics at 3 sites (GSTT, KCH and UHL), patient recruitment, patient assessments, processing of laboratory samples and data analysis. I also set-up and maintained a REMIRA database on ACCESS.

Adult RA patients diagnosed according to the 1987 revised ACR criteria were recruited into the REMIRA (REMission in RA) study. Inclusion criteria restricted disease duration to <10 years (as defined by date from diagnosis). We chose this as a cut-off as we were particularly interested in patients who were more likely to have had contemporary treatment of RA. To ensure all recruited study subjects were in stable LDA, we also included the following criteria: stable DMARDs therapy > 6 months and DAS28-ESR ≤ 3.2 for at least 1 month. Three centres across south London participated in this study: Guy's and St Thomas' Hospital, King's College Hospital and University Hospital Lewisham NHS Foundation Trusts. The study was approved by the local ethics committee and conducted according to the guidelines of the Declaration of Helsinki (REC:09/H0803/154, Wandsworth Research Ethics Committee). Local Research and Development (R&D) approval was obtained from

all sites. Written informed consent was obtained from all participants. Summary of REMIRA study in Appendix 11.17.

2.3.1 Clinical measures

2.3.1.1 Patient assessments

Clinical data including demographics, smoking, disease duration (as defined as from date of diagnosis) and treatment were collected. Extended 68-tender joint (TJC68) and 66 -swollen joint (SJC66) counts were performed (see appendix 11.5 – 12) for consent form, patient information leaflet and assessment form). For the evaluation of pain, fatigue, patients' as well as evaluators' global assessment of disease activity, 100mm visual analogue scales (VAS) were used. Early morning stiffness (EMS) was recorded in minutes. Erythrocyte Sedimentation Rate (ESR) was collected from routine clinical laboratory results. For the evaluation of remission, DAS28ESR, DAS28CRP, SDAI, CDAI and ACR/EULAR Boolean remission criteria were used. Patients were assessed 3 monthly for 1 year.

2.3.1.2 Patient reported outcome measures

Health Assessment Questionnaire Disability Index (HAQ-DI) ranged from 0-3 with higher scores indicating more disability. The Medical Outcomes Study 36-Item Short-Form Health Survey (SF36) assesses health-related quality of life. It comprises of 8 domains: physical function (PF), physical role (RP), bodily pain (BP), general health perception (GH), vitality (VT), social function (SF), emotional role (RE) and mental health (MH). These domains are then generated into two summary measures: the physical component score (PCS; including PF, RP, BP and GHP) and

the mental component score (MCS including VT, SF, RE and MH). The scores are transformed to have a mean with S.D. of 10. Both range from 0-100. EuroQol also known as EQ-5D is a standardized instrument for use as a measure of health outcome. There are 5 dimensions which are combined to generate a single index of quality of life range between -0.594 to 1. In addition, there is also a EQ VAS which is a 20cm vertical VAS that generates a self-rating of health-related quality of life. The Functional Assessment of Chronic Illness Therapy (FACIT-F, (Yellen et al. 1997)) is a 13 item questionnaire that assesses self-reported fatigue (range 0-52). With the SF36, EurQol and FACIT-F, the greater the number, the better the reported outcome. These were all measured 3-monthly for the length of the study. (see appendix for PROMs questionnaires).

2.3.2 Radiographic outcomes

2.3.2.1 Radiographs of hands and feet

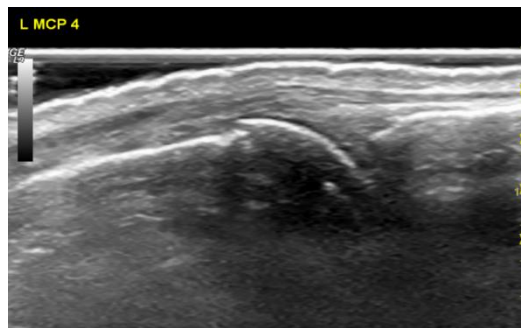
Posterior-Anterior conventional radiographs of the hands and feet were taken at baseline and 12 months. Erosive progression is defined as new erosions or worsening of existing erosions over 1 year.

2.3.2.2 Ultrasound assessments

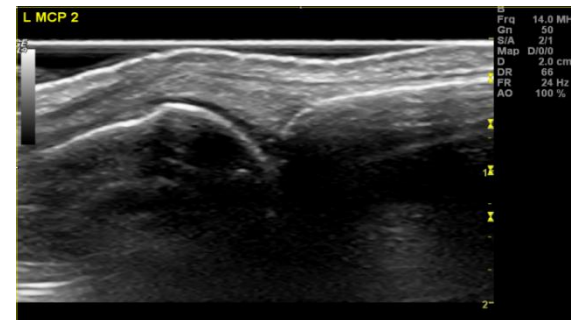
Ultrasound examination was carried out in all patients at baseline and 12 months. All sonographic assessments were performed using high-sensitivity ultrasound equipment (GE Logiq 9), with a 2D M12L transducer. Sonographic assessments were performed using a frequency of 14MHz, Gain 50, Depth 2.0cm, Frame Rate 24. When performing power doppler (PD) evaluation, frequency of 7.5 MHz and pulse repetition frequency was set between 500 to 800 Hz. The receiver gain

settings were systematically increased and decreased to achieve the highest gain without the appearance of artifacts. An experienced blinded sonographer (Dr Toby Garrood), without access to clinical or laboratory data, scanned 6 joints of each hand (1-5 Metacarpophalangeal joints and wrists) for both synovial hypertrophy (SH) and intraarticular PD signals (Ozgoçmen et al. 2008). All included joints were scanned for SH and PD in the dorsal aspect using longitudinal midline as in accordance with OMERACT guidelines (Wakefield et al. 2005). In addition, 2 other views of the wrists were obtained longitudinal (ulnocarpal and radiocarpal). MCPs and wrists are the most commonly used joints for ultrasound and we have therefore opted to use these joints. Due to limited time, we were unable to scan more number of joints. Grading of the scans were carried out retrospectively using saved images. Still were saved for grey scale and 3 second cine-loops were recorded for power doppler assessments. SH and PD were graded using a 4-grade semiquantitative scoring system from 0-3 according to the method developed by Wakefield (Wakefield et al. 2005). In SH, Grade 0 = no hypertrophy, Grade 1 = minimal: below the level of bony joint line, Grade 2 = above the level of bony joint line but without distension of joint capsule (forming concavity of the upper joint surface) and Grade 3 = severe: above the level of bony joint line with distension of joint capsule (forming convexity or flattening of upper surface). In PD, Grade 0 – no flow in synovium, Grade 1 – up to 3 single spots OR up to 2 confluent spots OR 1 confluent and 2 single spots, Grade 2 – more than grade 1 but <50% of gray scale area and Grade 3 – vessel signals >50% gray scale area (see Figures 2.1-2.8 for representative images of MCPs and all 3 views of the wrists). The total SH score and total PD score are the sums of the average of all 3 views of the each wrist plus each of the MCPs.

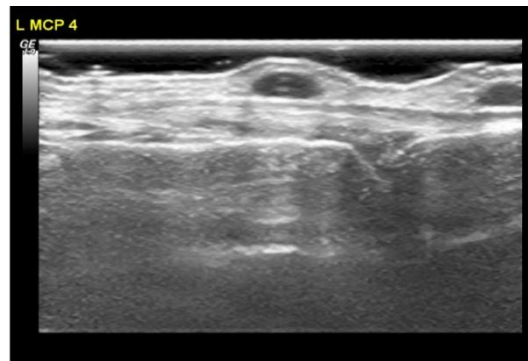
Grade 0



Grade 1



Grade 2



Grade 3

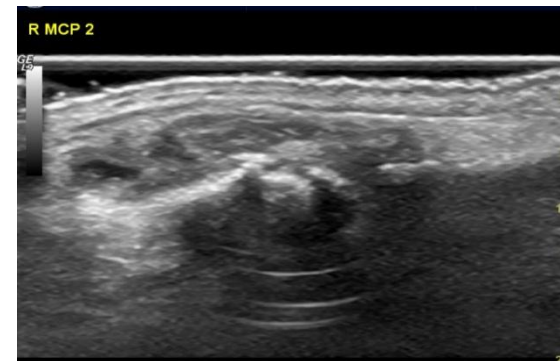
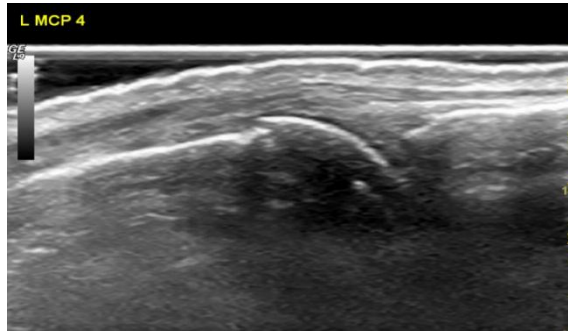
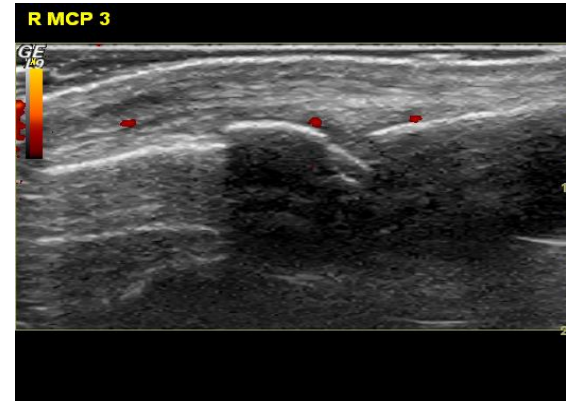


Figure 2-1: Representative images of SH of MCP SH (Synovial Hypertrophy - Grades 0-3)

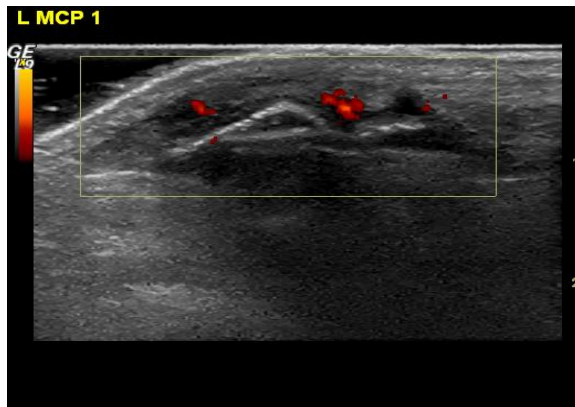
Grade 0



Grade 1



Grade 2



Grade 3

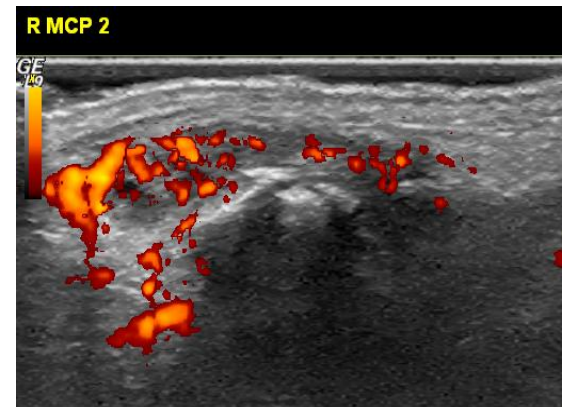
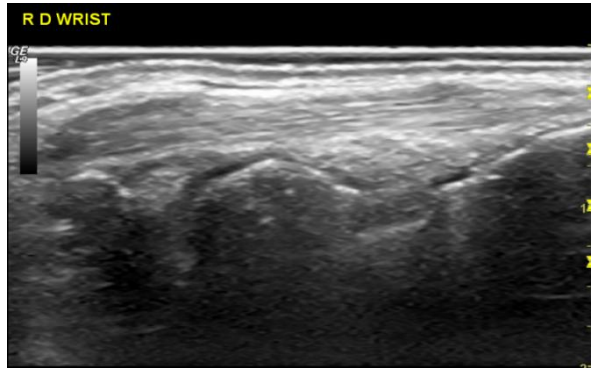
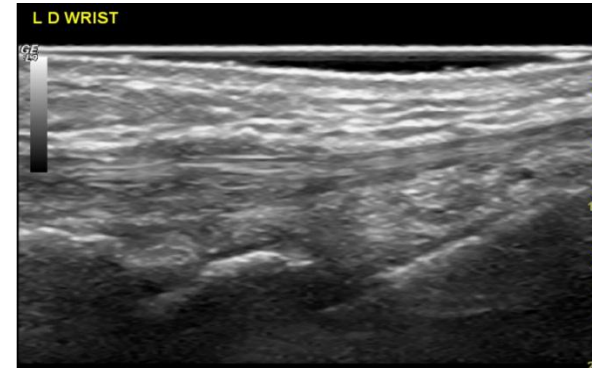


Figure 2-2 Representative Images of Grading of PD of MCP (Power Doppler Grades 0-3)

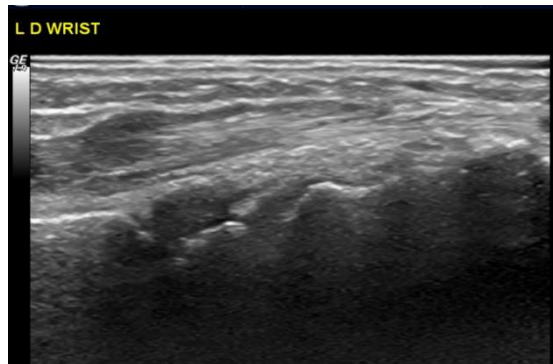
Grade 0



Grade 1



Grade 2



Grade 3

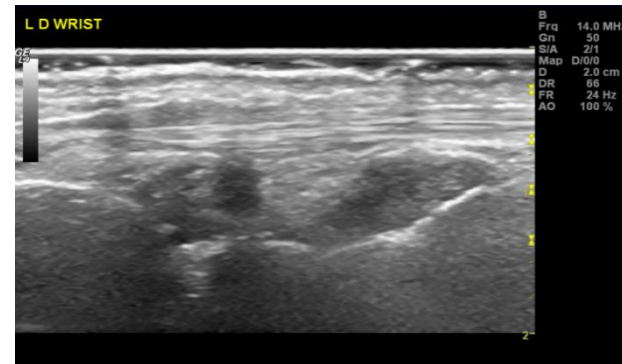
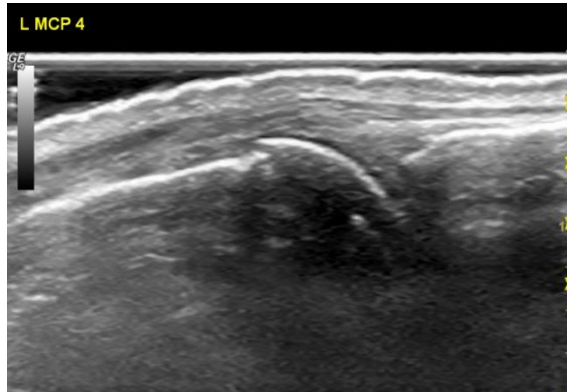
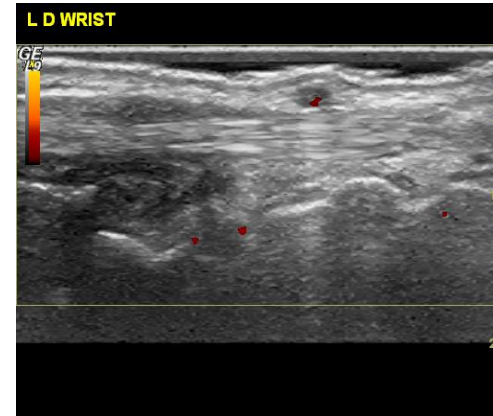


Figure 2-3 Representative Images of Grading of SH of the Dorsal Wrist (Synovial Hypertrophy - Grades 0-3)

Grade 0



Grade 1



Grade 2

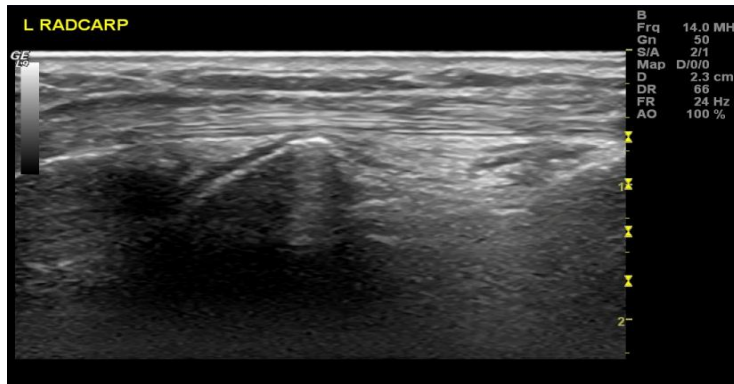


Grade 3

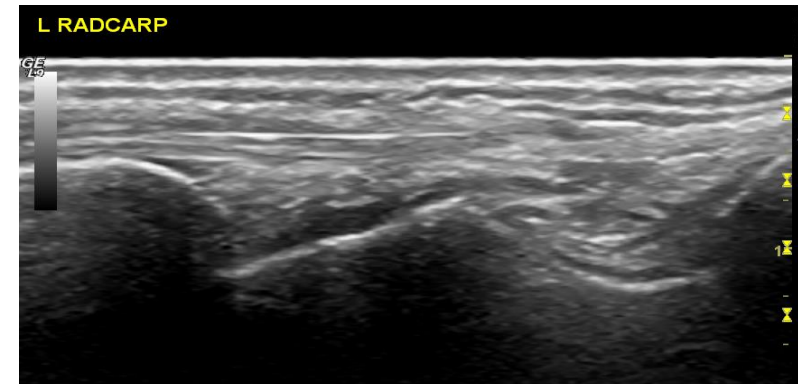


Figure 2-4 Representative Images of Grading of PD of the Dorsal Wrist (Power Doppler Grades 0-3)

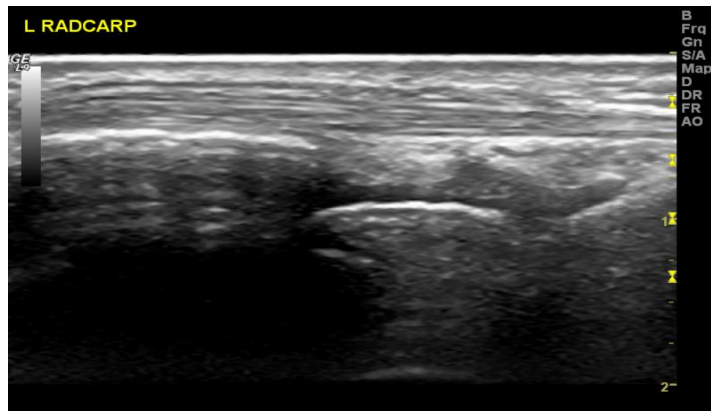
Grade 0



Grade 1



Grade 2



Grade 3

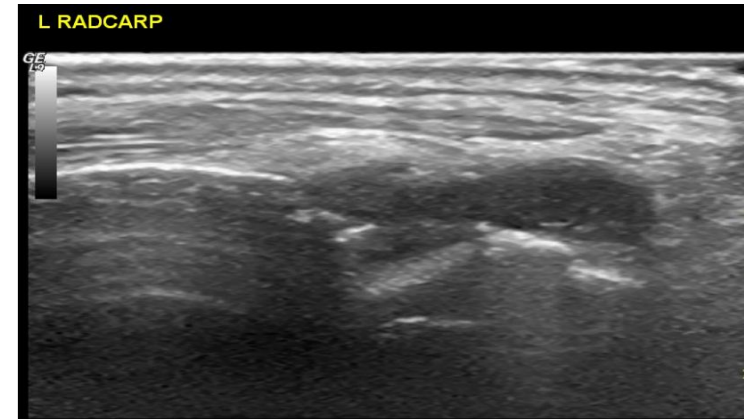
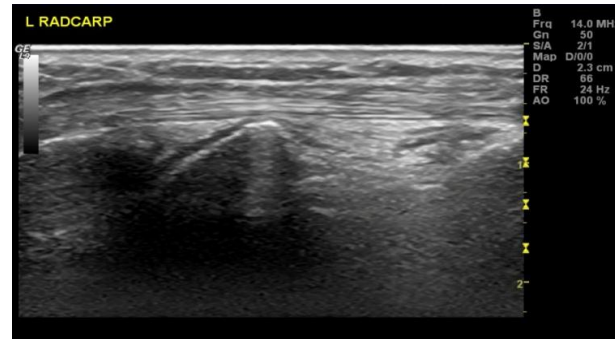
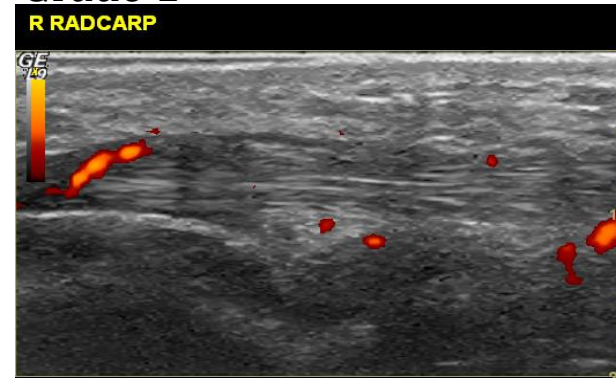


Figure 2-5 Representative Images of Grading of SH of Radiocarpal Joint (Synovial Hypertrophy - Grades 0-3)

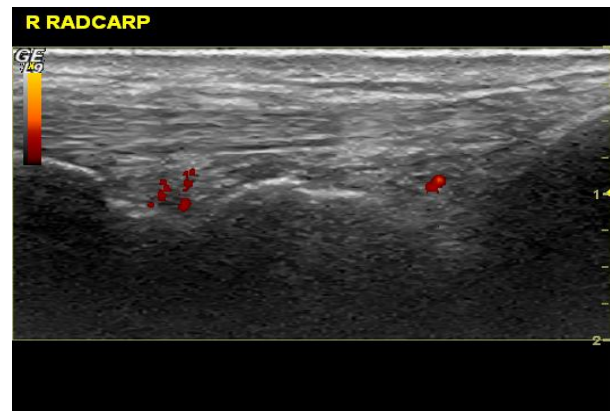
Grade 0



Grade 1



Grade 2



Grade 3

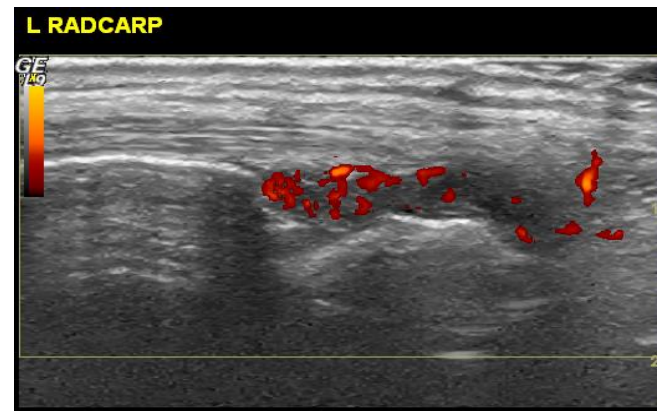
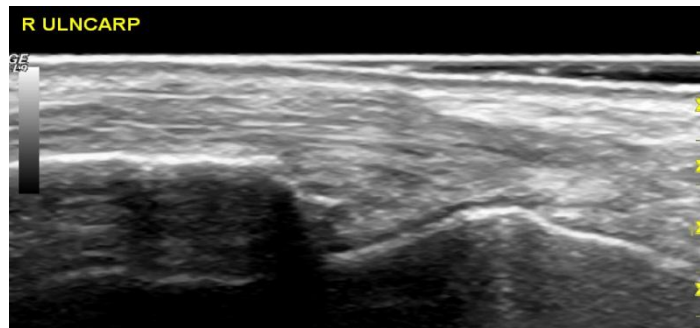


Figure 2-6 Representative Images of Grading of PD of Radiocarpal Joint (Power Doppler - Grades 0-3)

Grade 0



Grade 1

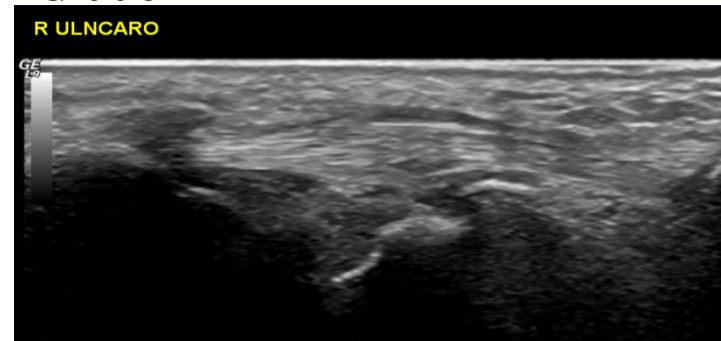
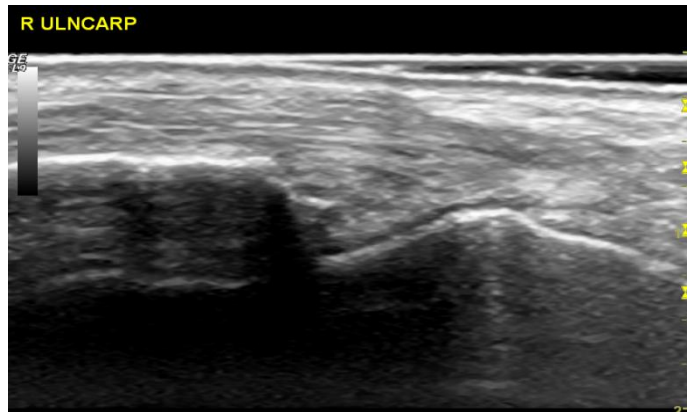


Figure 2-7 Representative Images of Grading of SH of Ulnocarpal Joint (Synovial Hypertrophy - Grades 0-3)

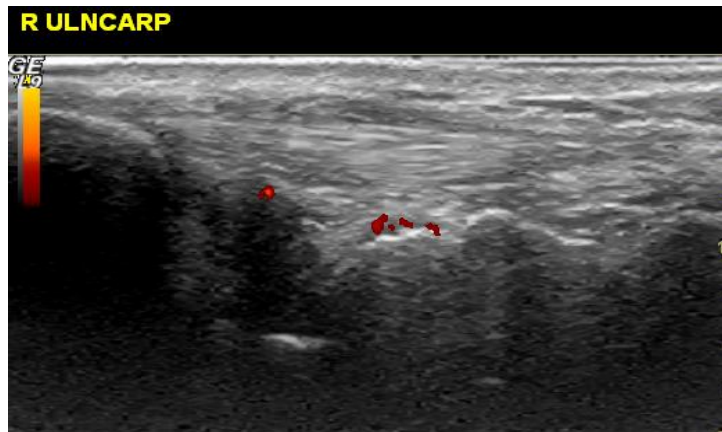
Grade 0



Grade 1



Grade 2



Grade 3

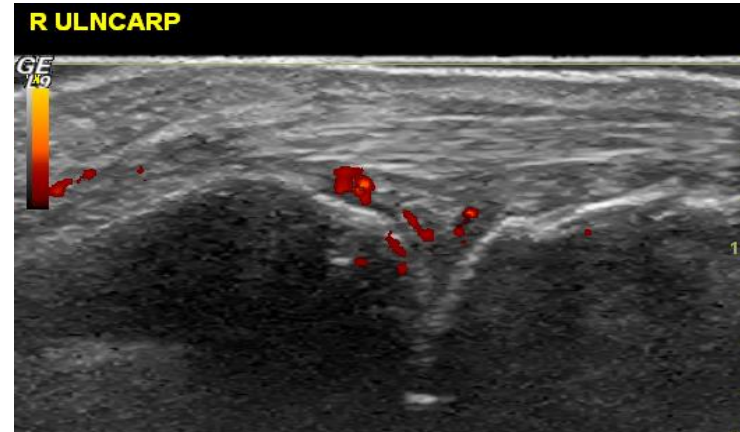


Figure 2-8 Representative Images of Grading of PD of Ulnocarpal Joint (Power Doppler - Grades 0-3)

2.3.3 Blood sampling

2.3.3.1 At baseline visit

- 4 x sodium heparin GREEN TOP tubes 40ml
- 2 x plain BRICK RED TOP tubes for serum 16ml
- 2 x PAX gene tube for whole blood RNA 8ml
- 2 x Tempus tubes for whole blood RNA 6ml

2.3.3.2 At follow-up: 3, 6 and 9 month visit

- 4 x sodium heparin GREEN TOP tubes
- 2 x plain BRICK RED TOP tubes for serum
- 2 x Tempus gene tube for whole blood RNA

2.3.3.3 At end of study: 12 month visit

- 4 x sodium heparin GREEN TOP tubes - for PBMCs
- 2 x PAX gene tube for whole blood RNA
- 2 x Tempus tubes for whole blood RNA
- 2 x plain BRICK RED TOP tubes for serum
- 1 x Paxgene DNA tube

2.3.4 Protocol for blood sample processing

The bloods samples were all processed on the same day as sampling. Courriers were used to transport samples from KCH (King's College Hospital) and UHL (University Hospital Lewisham) to the lab. The protocols used for blood sampling and processing are summarized below:

2.3.4.1 PBMC isolation and freezing

Materials required

- 2 x 50ml Leucosep tubes
- Hank's Balance Salt Solution (HBSS, Sigma)
- Sterile Phosphate Buffered Saline (PBS, Fisher)
- Lymphoprep (Axis Shield)
- Cell culture grade DMSO (Sigma)
- RNeasy Protect cell (Qiagen)
- AB serum (PAA)
- Coolcell freezing containers (VWR)

Protocol

1. Dilute blood in sterile HBSS (1:1) in 50 ml Corning tubes
2. Aliquot 15ml Lymphoprep in all Leucosep tubes
3. Centrifuge Leucosep tubes for 30 seconds at 1000g at RT
4. Pour 35ml of diluted blood into each of 2 X Leucosep tubes
5. Spin in centrifuge (10min, 1000g, no brake, RT)
6. Check for interphase
7. Using Pastuer pipette, remove plasma layer fraction up to a minimum remnant of 5 to 10 mm above the interphase to remove contamination of the enriched cells with platelets.
8. Harvest the enriched cell fraction using a pastuer pipette or by pouring the supernatant above the porous barrier into another tube
9. Wash with ice-cold Phosphate-buffered saline (PBS) – make up to 50ml

10. Spin in centrifuge (10 min, 1600rpm, brake, RT)
11. Check for cell pellet and discard supernatant
12. Wash pellet in sterile ice-cold 10ml PBS again
13. Spin in centrifuge (10 min, 1200rpm, brake, RT)
14. Add 2 ml ice-cold PBS to cell pellet
15. Count cells using cell counter
16. Take out 5×10^6 cells for RNA (see RNA section for further instructions)
17. Add 3mls of PBS to cell suspension (making it up to 5ml)
18. Spin down cells in a cold centrifuge at 1200 rpm. Decant supernatant and flick tube gently to loosen cell pellet.
19. Place freezing medium – (i) neat filtered AB serum and (ii) mix of 80% filtered AB serum + 20% high purity DMSO – on ice for at least 10 minutes. Place cryovials also on ice.
20. Add 500ml neat AB per $0.5-1 \times 10^7$ cells. The cells were not frozen any denser than this. Pipette gently to minimize shear force 3 times and avoid bubbles.
21. Add equal volume of AB serum/DMSO and mix slowly with each drop; add dropwise over 1-2 minutes, repeatedly swirling tube gently to mix.
22. Pipette 1ml into cryovials. Work quickly if there are many cryovials.
23. Transfer to CoolCell and into -80°C freezer for 24 hours then to Liquid nitrogen.

2.3.4.2 Protocol for storing PBMC RNA

1. 1.5×10^6 PBMCs were used for RNA storage.
2. Add 5 volumes of RNeasy Protect Cell Reagent (Qiagen) to 1 volume of cell-culture medium or storage solution.
3. Mix by shaking, pipetting, or vortexing.

4. Note: The medium or storage solution must not exceed 1 ml.

2.3.4.3 Protocol for storing whole blood RNA and DNA

1. PAXgene and Tempus tubes contain buffer to ensure immediate lyses of the blood cells and stabilisation of the RNA.
2. Incubate the blood in vacutainers at room temperature for 2 hours
3. Store tubes at -20°C for at least 24 hours
4. Transfer to -80°C until further processing

2.3.4.4 Protocol for serum

1. Blood in the vacutainers are kept in the fridge whilst awaiting processing.
2. Spin in vacutainer at 1200rpm, 10min.
3. Harvest serum
4. Spin serum repeatedly until no red cell pellet is visible (1200rpm, 10min).
5. Freeze aliquots in -80°C

2.3.5 Serum biomarkers

All 14 biomarkers were measured by Crescendo Bioscience laboratories. The development of the MBDA score has been discussed in the introduction (see section 1.7.8). The concentrations of 12 serum proteins—serum amyloid A (SAA), IL-6, TNF receptor superfamily member 1A (TNF-R1), VEGFA, MMP1, human cartilage glycoprotein 39 (YKL40), MMP3, epithelial growth factor (EGF), vascular cell adhesion molecule 1 (VCAM1), leptin, resistin and CRP—were measured by customized immunoassays, quantified on a Sector Imager 6000 (Meso Scale

Discovery, Gaithersburg, MD, USA) and transformed to the power 0.1 to achieve approximately normal distributions.

CXCL10 and Calprotectin were measured by using commercially available ELISA kits according to the manufacturers' protocols. The Calprotectin assay was from Buhlmann (MRP 8/14 ELISA Product Code EK-MRP8/14). For CXCL10, a modified version of the R&D Systems Human CXCL10/IP-10 Quantikine ELISA (Product Code: DIP100) was used. For this ELISA assay, in-house manufactured pre-diluted standards and controls were used. A 2-fold dilution of samples was carried out. The rest of the procedure was performed per the manufacturer's protocol.

2.4 Statistical analysis

Statistical analyses are described within each chapter separately.

3 Remission in Early Rheumatoid Arthritis: Systematic Review

3.1 Introduction

The advent of intensive treatment regimens has made remission a realistic treatment goal in early rheumatoid arthritis (RA) (Boers et al. 1997, Choy et al. 2008). These intensive treatments including combinations of disease modifying anti-rheumatic drugs (DMARDs) and DMARDs with biological therapies such as tumour necrosis factor (TNF) inhibitors (Allaart et al. 2006, Grigor et al. 2004, Breedveld et al. 2006, Mottonen et al. 1999) and they are associated with higher rates of remission. These regimes are now part of the standard treatment of care in RA with remission as the main treatment goal. Despite remission being a key goal of RA treatment, its frequency on treatment has not been evaluated methodically.

Several classification criteria have been developed for remission. Some criteria use categorical descriptions of remission whilst others are continuous composite measures are also used to define remission. These have been described in the introduction (see section 1.11). Radiological progression is not considered in these remission criteria in spite of its importance in long-term disability (Scott et al. 2000).

The first step of the thesis was therefore to systematically review observational and randomised controlled trials in early RA with three aims. Firstly, to identify the differences in the frequency of remission dependant on the criteria by which it is judged. Secondly, to determine how the frequency of remission is influenced by

different treatment strategies. Finally, to review the effects of remission on radiological outcomes.

3.2 Methods

3.2.1 Search Terms

Pubmed, EMBASE and Medline were searched using the following search terms: Early Rheumatoid Arthritis or Early RA combined with Remission, Treatment, anti-TNF or DMARD. The search was limited to 1996 – 2008, English and Clinical trials.

3.2.2 Selection criteria

Studies were selected for inclusion using the following criteria:

1. Randomised Controlled Trials or Observational Studies
2. Patients fulfilled the ACR classification of RA
3. Disease duration less than 3 years of diagnosis.
4. Remission used as an outcome measure
5. Enrolled more than 40 patients

3.2.3 Outcomes

DAS (and its modifications) or ACR (and its modifications) remissions were used as the clinical outcome measure. Radiological outcomes of patients in remission were also assessed.

3.2.4 Quality of trials

The quality of the trials was judged using the Jadad Scoring System (Jadad et al. 1996). The Jadad scoring system, also known as The Jadad scale or the Oxford quality scoring system, is a procedure to independently assess the methodological quality of a clinical trial. It consists of 3 elements: Randomisation, blinding and withdrawals/dropouts. It ranges from 0-5, 0 being the weakest and 5 being the strongest. See appendix 11.1 for the calculations.

3.2.5 Data Extraction

I had carried out the data extraction. For the purpose of publication, Dr Ian Scott acted as the second observer. Studies were assessed for eligibility and extracted data on year of publication, population source, study design, study size and follow-up period. When there were differences between observers, they reviewed the papers together and came to a joint conclusion.

3.2.6 Statistical Analysis

Data from all studies were analysed descriptively. RCTs were analysed using Review Manager 4.2.8 (Cochrane Collaboration, Oxford, United Kingdom). The random effects odds ratio (OR) model based on DerSimonian and Laird's method was used to estimate the pooled effect sizes (DerSimonian, Laird 1986); this gives more equal weighting to studies of different precision in comparison to a simple inverse variance weighted approach, thereby accommodating between study heterogeneity. It was reported with 95% confidence intervals (CI). For all meta-analyses, Cochran's Chi-Squared test was performed to assess between study heterogeneity and quantified the I^2 statistic (Hardy, Thompson 1998, Higgins et al.

2003). We considered a p-value less than 0.05 as significant. The number needed to treat (NNT) was calculated and reported with 95% CI.

In RCTs with more than one 'control' arm or 'treatment' arm, the arm with the best outcome was selected for analysis.

3.2.7 Update In 2014

The initial systematic review was undertaken in 2009 and 2010 and was published in 2010 (Ma et al. 2010). In the ensuing four years several new studies have been published on remission in RA. In order to retain the original peer-reviewed published systematic review and to ensure this thesis is up to date, details of these new studies have been collated as an addendum to the chapter. They used identical methods, although the dates for the search were extended.

3.3 Results

3.3.1 Study Selection

1660 citations were identified for review, 52 were evaluated in detail and 37 studies were included in the final analysis. These comprised 17 observational studies and 20 RCTs (Figure 3.1). The baseline characteristics of the observational studies and RCTs are described in Tables 3.1 and 3.2 respectively. From the available data, the patients enrolled into the RCTs appeared to have higher disease activity.

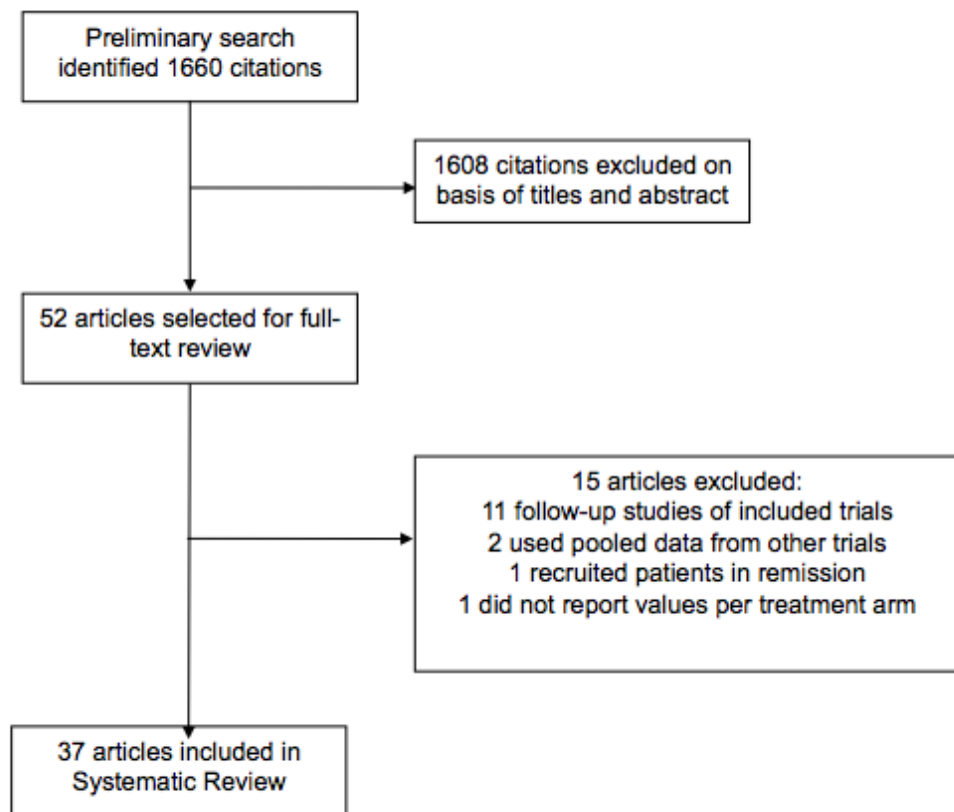


Figure 3-1: Search Strategy of the systematic review

The 17 observational studies (Table 3.1) followed patients for 2-10 years: 16 reported end point remissions and 1 reported remissions over 6 months at any point during follow-up. 4762 patients entered these observational studies (3653 completing full follow-up); 972 (27%) achieved remissions.

The 20 RCTs (Table 3.2 and 3.3) followed patients for 1-3 years. Their average Jadad score was 3.5 (range 1-5). 19 RCTs reported end-point remissions and 1 reported remission at any time point. Four trials evaluated DMARD monotherapies (2 monotherapy vs placebo/NSAID; 2 different monotherapies). 13 trials compared monotherapy with combination therapies. 3 trials reported different combination strategies. 4290 patients entered these trials; 1312 (31%) achieved remissions.

Table 3-1 Patient Characteristics and Remission in Observational Studies

Table shows remission rates at the end of the study *remission over 6 months at any point, Results are mean values unless denoted by suffix "i" indicating median data; ii DAS

Study	Year	Remission	Disease Duration (months)	Age (years)	Female (%)	RF+ve (%)	ESR	DAS28	Follow-up (months)	DMARDs	Number at entry	Number at f/u	Remission (at study end)
Prevoo et al.,	1996	ACR	<12	55 ⁱ	63	78	-	-	72	Monotherapy	227	49	15 (31%)
Eberhardt and Fex	1998	ACR	<24	-	63	75	29	-	60	Monotherapy	183	176	37 (20%)*
Young et al	2000	ACR	8	-	66	63	-	-	60	Monotherapy	941	732	94 (13%)
Makinen et al	2005	ACR	5	56	61	54	-	-	60	Monotherapy	127	111	19 (17%)
Möttönen et al	1996	ACR	<24	46	75	63	30 ⁱ	-	72	Combination	142	142	45 (32%)
Lindqvist et al	2002	ACR	<24	-	63	75	-	-	120	Combination	183	163	30 (18%)
Sanmarti et al	2003	ACR	<24	52	78	-	45	5.8	12	Combination	65	60	12 (20%)
Fransen et al	2004	ACR	<12	55	66	76	-	-	72	Combination	424	77	9 (12%)
Cantagral et al	1999	DAS	<12	-	-	71	-	-	24	Not stated	108	108	15 (14%)
Tengstrand et al	2004	DAS	<12	57	64	58	-	5.1	24	Monotherapy	844	844	279 (33%)
Vázquez et al	2007	DAS	<24	55	81	74	40	5.7	24	Monotherapy	115	105	34 (32%)
Khanna et al	2007	DAS	<14	51	-	100	43	5.5	24	Monotherapy	200	101	33 (33%)
Gossec et al	2004	DAS	<12	51	73	81	40	4.1 ⁱⁱ	60	Combination	191	165	38 (23%)
Forslind et al	2007	DAS	≤12	58	64	60	-	5.3	60	Combination	698	608	234 (39%)
Proudman et al	2007	DAS	<24	56	76	61	42	5.3	36	Combination	61	52	28 (54%)
Sanmarti et al	2007	DAS	<24	55	81	74	40	5.7	24	Combination	115	105	34 (32%)
Machold et al	2007	DAS	≤3	51	75	-	-	-	36	Combination	138	55	16 (29%)

Table 3-2 Patient Characteristics in the Clinical Trials.
Results are mean values. RF = rheumatoid factor

Study	Year	Control					Treatment				
		Age (years)	Female (%)	RF+ve (%)	ESR	DAS28	Age (years)	Female (%)	RF+ve (%)	ESR	DAS28
Monotherapy											
Eberhardt et al	1996	53	63%	80%	36	-	50	52%	67%	32	-
Rau et al	1997	54	60%	68%	41	-	57	72%	54%	41	-
Van Jaarsveld et al	2000	56	69%	67%	42	-	57	64	65	41	-
Choy et al	2002	58	74%	54%	-	DAS 5.3	57	76%	58%		5
Monotherapy vs Combination Therapy											
Boers et al	1997	49	52%	72%	-	-	50	66%	78%	-	-
Möttönen et al	1999	48	66%	66%	39	-	47%	58%	70%	37	-
Proudman et al	2000	50	55%	79%	31.4	5.1	51%	65%	80%	39.1	5.4
Ferraccioli et al	2002	59	86%	55%	43	-	59	86%	73%	52	-
Gerards et al	2003	51	70%	97%	46	-	53	62%	93%	53	-
Wassenberg et al	2005	50	65%	47%	40	-	53	75%	43%	44.5	-
St Clair et al	2004	50	75%	71%	43	6.7	50	68%	73%	44	6.7
Svensson et al	2005	59	63%	66%	-	5.4	51	65%	66%	-	5.28
Allaart et al	2006	54	68%	67%	-	DAS44 4.5	54	66%	64%	-	DAS44 4.3
Breedveld et al	2006	52	74%	-	-	6.3	52	72%		-	6.3
Choy et al	2008	54	67%	66%	-	5.8	55	67%	72%	-	5.6
Emery et al	2008	52	73%	-	49	6.5	51	74%	-	47.8	6.5
Hetland et al	2006	51	70%	59%	27	5.5	53	64%	70%	28	5.3
Combination vs Combination therapy											
Verstappen et al	2007	53	66%	62	39	-	54	69%	66%	36	-
Saunders et al	2008	55	79%	72%	45	6.9	55	76%	69%	36	6.8
Verschueren et al**	2008	55	65%	52%	-	DAS28 CRP 4.76	45	63%	79%	-	DAS28 CRP 5.28

Table 3-3 Remission in Control and Treatment Arms of Clinical Trials.

Study	Year	Disease duration (mths)	Follow-up (mths)	Remission	Control			Treatment		
					Cases	Treatment	Remission Rate	Cases	Treatment	Remission Rate
Monotherapy										
Eberhardt et al	1996	24	24	ACR derivative	22	Placebo	5 (12%)	21	D-Penicillamine	4 (12%)
Rau et al	1997	16	12	ACR derivative	87	MTX	10 (12%)	87	GSTM	21 (24 %)
Van Jaarsveld et al	2000	<12	24	ACR derivative	107	HCQ	29 (27%)	105	MTX (short lag)	25 (24%)
Choy et al	2002	<12	12	DAS28	55	Diclofenac	0	62	SSZ	0
Monotherapy vs Combination therapy										
Boers et al	1997	<24	12	ACR	76	SSZ	19 (24%)*	79	SSZ/MTX/Pred	24 (32%)*
Möttönen et al	1999	<24	24	ACR	98	SSZ or MTX	18 (18%)	97	MTX/SSZ/HCQ/Pred	36 (37%)
Proudman et al	2000	<12	12	ACR	42	SSZ	4 (10%)	40	MTX/CSA/IA Methylpred	5 (13%)
Ferraccioli et al	2002	16	36	ACR	42	SSZ	3 (7%)	42	MTX/CsA	4 (9%)
Gerards et al	2003	<36	12	ACR	60	CsA	4 (7%)	60	CsA/MTX	6 (10%)
Wassenberg et al	2005	<24	24	ACR	86	DMARD	8 (9%)	80	DMARD/Pred	13 (16%)
St Clair et al	2004	<36	12	DAS28	245	MTX	37 (15%)	325	MTX/Infliximab	101 (31%)
Svensson et al	2005	<12	24	DAS28	126	DMARD	42 (33%)	116	DMARD/Pred	65 (56%)
Allaart et al	2006	<12	24	DAS44	126	DMARD	58 (46%)	128	MTX/Infliximab	54 (42%)
Breedveld et al	2006	<36	24	DAS28	257	MTX	64 (25%)	268	MTX/Adalimumab	131 (49%)
Choy et al	2008	<24	24	DAS28	117	MTX	21 (18%)	116	MTX/CsA/Pred	32 (28%)
Emery et al	2008	<24	12	DAS28	263	MTX	73 (28%)	265	MTX/Etanercept	132 (50%)
Hetland et al	2006	<6	12	DAS28/ACR	68	MTX/IAsteroid	23 (34%)/19 (28%)	69	MTX/CsA/IAsteroids	30 (43%)/24 (35%)
Combination vs Combination therapy										
Verstappen et al,	2007	<12	24	ACR derivative	148	Usual MTX/CsA	55 (37%)	151	Intensive MTX/CsA	76 (50%)
Sauders et al	2008	Mean 12	12	DAS28	44	Step up	21 (45%)	47	Parallel	16 (33%)
Verschueren et al**	2008	<12	12	DAS28	17	Step up	No values	46	Step down	No values

Cases at end of follow-up, *achieving remission at some point during follow-up (probable and definite remissions included), ** Not randomised. GSTM = Gold Sodium Thiomalate, MTX = Methotrexate, HCQ = Hydroxychloroquine, SSZ = Sulphasalazine, Methylpred = Methylprednisolone, Pred = Prednisolone, CsA = Ciclosporin A ¶ = inclusion criteria of symptoms < 5 years but mean disease duration <12 months (SD <12 months).

3.3.2 Remissions In Observational Studies

Eight studies reported remissions using 1986 ACR criteria; 5 excluded fatigue and 1 used low levels of pain (<10mm on a 100mm VAS scale). The overall remission rate was 261/1501 (17%). The maximum disease duration ranged from 5 to 24 months. The mean remission rate of patients with <1 year disease duration was 18% and < 2 years disease duration was 23%. The follow-up period ranged from 1 to 10 years. When these were sub-divided into groups (<3 years, <6 years and >6 years), the mean remission rates were similar (20%, 21% and 18% respectively).

Four studies reported ACR remission rates in patients receiving only DMARD monotherapies; 165/1068 (15%) of these patients achieved remission. Four studies reported ACR remission in patients also receiving combination therapies; 96/442 (22%) patients achieved remission.

Nine studies used DAS based remission criteria: 2 used $\text{DAS} \leq 1.6$ and 7 $\text{DAS28} \leq 2.6$. The overall rate of remission was 711/2143 (33%). The maximum disease duration ranged from 3 to 24 months. The mean remission rate of patients with <1 year disease duration was 29% and <2 years disease duration was 36%. The follow-up period ranged from 1 to 6 years. When these were sub-divided into groups (<3 years and <6 years), the mean remission rates were 32% and 31% respectively. Three studies reported DAS remissions in patients receiving only DMARD monotherapies; 328/1057 (31%) achieved remission. Five studies reported remissions in patients receiving

combination therapies; 350/985 (36%) achieved remission. One study did not describe the treatments used (Cantagrel et al. 1999).

3.3.3 Remissions In Clinical Trials Of DMARD Monotherapies

Four RCTs evaluated remissions with DMARD monotherapies: one compared DMARD (D-Penicillamine) with placebo; one compared SSZ with NSAIDs; and two compared different DMARD monotherapies. Three RCTs used categorical remission criteria based on ACR remission (ACR derivative). 5/22 (12%) achieved remissions with placebo therapy. There were 89/469 (19%) patients in remission using DMARD monotherapy. One RCT used DAS-based remission criteria but did not identify remissions with DMARD monotherapy or NSAIDs (Choy et al. 2002).

3.3.4 Remissions In Clinical Trials Of Combination Therapies

Thirteen RCTs compared DMARD monotherapy with combination DMARD therapy (including biologics). Six used ACR-based remission criteria: two excluded fatigue; one excluded morning stiffness. They reported 75/472 (16%) patients achieved remissions with monotherapies and 112/467 (24%) with combination therapies. Maximum disease durations were from 12-36 months. The mean remission rate of patients with <1 year disease duration was 19% with monotherapies and 24% with combination therapies, < 2 year disease duration was 15% with monotherapies and 24% with combination therapies and <3 year disease duration was 7% with monotherapy and 10% with combination therapy. The follow-up period ranged from 1-3 years. When these were sub-divided into groups (<1 years, <2years and <3 years),

the mean remission rates were similar (17%, 14%, 7% with monotherapies and 23%, 27% and 9% with combination therapies). Meta-analysis (Table 3.4, Figure 3.2) showed the random effects OR for remission with combination therapies compared with monotherapies was 1.69 (95% CI 1.21, 2.36). There was no evidence of significant heterogeneity. The NNT was 12 (95% CI 8, 33).

Seven RCTs used DAS remission criteria. 318/1202 (26%) patients achieved remissions with monotherapies and 545/1287 (42%) with combination therapies. The maximum disease duration ranged from 6 to 36 months. The mean remission rate of patients with <1 year disease duration was 26% with monotherapies and 41% with combination therapies, <2 year disease duration was 40% with monotherapies and 49% with combination therapies and <3 year disease duration was 22% with monotherapy and 39% with combination therapy. The follow-up period ranged from 1 to 2 years. When these were sub-divided into groups (<1 years and <2 years), the mean remission rates were 26% and 31% respectively with monotherapies and 41% and 44% respectively with combination therapies). Meta-analysis showed the random effects OR for remission with combination therapies compared with monotherapies was 2.01 (1.46, 2.78) with DAS remissions criteria. There was significant heterogeneity within the studies. The NNT was 6 (95% CI 5, 8). One trial reporting DAS and ACR remissions was included in both ACR and DAS remission analysis (Hetland et al. 2006). The effects of steroids, anti-TNF therapy, combination DMARD therapies and tight-control regimes were also investigated by using meta-analysis (table 3.4). The random OR were similar in all subgroups (1.51 – 2.23).

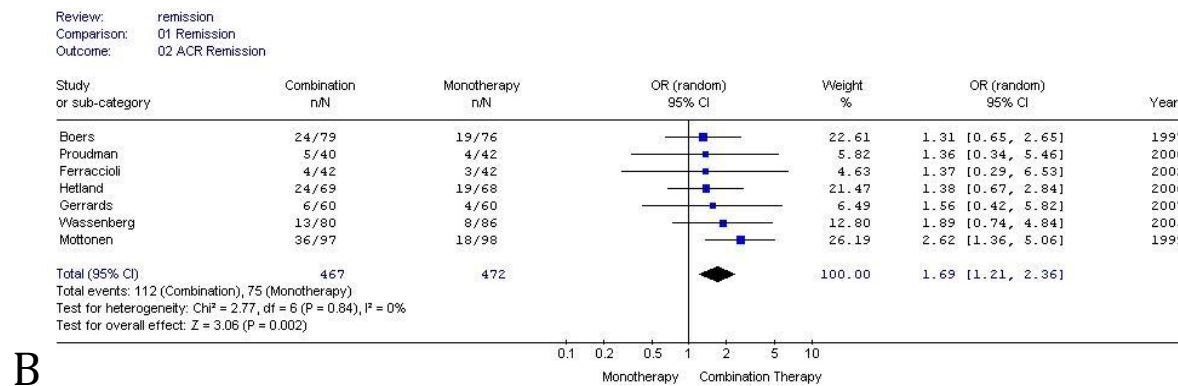
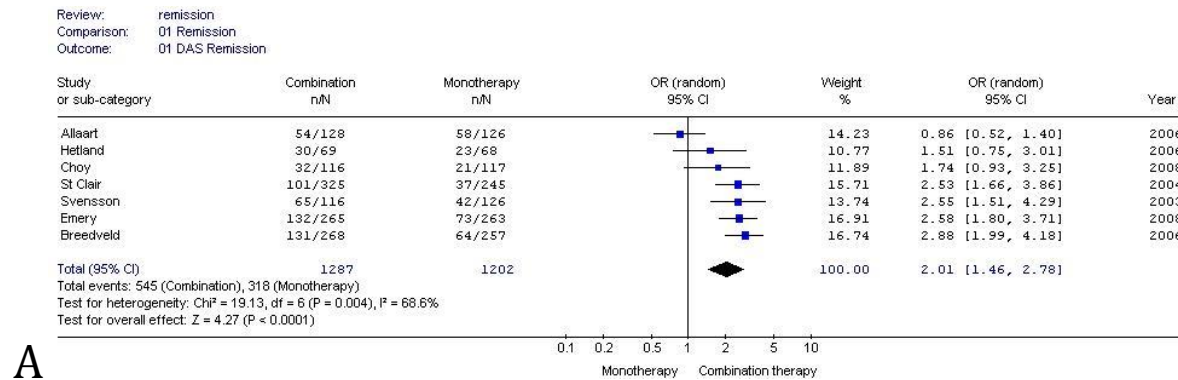


Figure 3-2: Forrest plot showing the meta-analysis of RCTs comparing combination treatment and monotherapies
(A) DAS remission criteria; (B) ACR remission criteria, n = number of patients in remission, N = number of patients in treatment arm

Table 3-4 Meta-Analysis of Remission Rates in RCTs
Comparing Monotherapy and Combination therapy

Sub-groups	Studies	Monotherapy		Combination Therapy		Random OR (95% CI)	Chi-squared	p value
		Cases	Remission	Cases	Remission			
DAS remission	7	1202	318	1287	545	2.01 (1.46, 2.78)	19.1	p = 0.004
ACRRemission	7	472	75	467	112	1.69 (1.21, 2.36)	2.8	p = 0.84
Steroids	3	328	91	315	132	1.95 (1.39, 2.73)	1.99	p >0.05
Anti-TNF	4	928	226	1383	390	2.05 (1.26, 3.34)	17.18	p = 0.0006
Combination DMARDs	10	841	200	953	294	1.51 (0.99, 2.31)	28.33	p = 0.0008
Tight control regimes	2	246	95	248	142	2.23 (1.26, 3.97)	2.39	p > 0.05

Three trials reported different combination strategies. One trial compared step-up and step-down combinations regimens but was not randomised (Verschuere, Esselens & Westhovens 2008); remission rates were similar with both treatments but no values were reported. Two RCTs reported ACR or DAS-based remissions in 33-50% of patients. Remission by any criteria occurred in 168/395 (43%) patients.

3.3.5 Remissions And Radiological Progression

Four observational studies reported radiological outcomes in patients in remission (Table 3.5). All showed some radiological progression (19%-54% patients over 3-5 years) using varying radiological assessment methods. Three studies compared erosive progression in patients achieving remission to other cases: one study (Machold et al. 2007) reported lower erosive progression with lasting remission defined as DAS28 < 2.6 for > 1 year (19% vs 72%); two studies found no differences.

Two RCTs reported the effects of remission on radiological outcomes (Allaart, Breedveld & Dijkman 2007, Svensson et al. 2005). Both showed less radiological progression with combination treatments compared to monotherapies in patients in remission (Table 3.6).

Table 3-5 Radiographic Outcomes In Observational Trials In Remission

*Lasting remission = DAS28 < 2.6 for > 1 year

Study	Year	Remission	Numbers at follow-up	Radiographic outcome
Makinen et al	2005	ACR	111	Radiological remission (no new/increased erosions) at 5 years in 66 patients (55%)
Vázquez et al	2007	DAS	105	Increase in Larsen score > 4 at 2 years: Remission 9/34 (27%) No remission 23/71 (34%)
Proudman et al	2007	DAS	61	Increase in erosion score at 3 years: Remission 15/28 (54%) No remission 14/24 (58%)
Machold et al	2007	DAS	55	New erosions over 3 years: Lasting remission* 3/16 (19%); No lasting remission 28/39 (72%).

Table 3-6 Summary of Radiographic Outcomes in RCTs in Remission

Study	Year	Monotherapy	Combination Therapy
Svensson et al	2005	Median change in Larsen Score at 2 years Remission 2.5 (IQR 0.5-8.0)	Median change in Larsen Score at 2 years Remission 1.0 (IQR 0-3.5)
Allaart et al	2006	Continuous Remission 25% damage progression	Continuous Remission 3% damage progression

3.4 Addendum: Studies Published Since 2010

3.4.1 Explanation

As outlined in the methods section (see 3.2.7), since the systematic review was completed and published, several new studies have appeared. These are summarized in this addendum. The methods used in this update were identical to those in the main review. As the initial review included studies published until the end of 2008 in this update studies were included that were published between January 2009 and December 2013.

3.4.2 Studies Identified

The update identified 77 new citations and 19 these were selected for review. 11 were excluded as they did not fulfill selection criteria: 6 studies had <50 patients within the study (Kita et al. 2012, Bejarano et al. 2010, Sakellariou et al. 2012, Benbouazza et al. 2012, Picchianti Diamanti et al. 2012), 4 were follow-up reports of previously included studies (Rantalaiho et al. 2010b, Rantalaiho et al. 2010a, Rantalaiho et al. 2009, Svensson, Hafstrom 2011), one included undifferentiated arthritis patients (Gremese et al. 2013) and one RCT did not report remission rates per treatment arm (Schipper et al. 2011). A further 4 RCTs were identified through hand searching (Soubrier et al. 2009, Moreland et al. 2012, Detert et al. 2013, Kavanaugh et al. 2013).

3.4.3 Observational Studies

Two new observational studies were identified (Sagawa et al. 2011, Benbouazza et al. 2012). These are summarized in Table 3.7. Both studies included the use of DMARD combinations. DAS28 remission rates ranged from 35% to 44%. This is higher than the studies in the original systematic review (33%). Sagawa et al (Sagawa et al. 2011) reported the highest remission rate but the drop-out rate was very large at the end of the 24 months period. This makes the results difficult to interpret.

Table 3-7 Remissions In Observational Studies (2009-2013)

Study	Year	Remission	Disease Duration (months)	Age (years)	Female (%)	RF+ve (%)	ESR	DAS28	Follow- up (months)	DMARDs	Number at entry	Number at f/u	Remission (at study end)
Sagawa et al	2010	DAS28	20	52	84	-	47	4.90	24	Combination	81	16	7 (43.8%)
Benbouazza et al	2011	DAS28	<12	47	89	63	57	6.90	24	Combination	51	45	16 (34.8%)

3.4.4 Randomised Controlled Trials

There were 6 new RCTs reporting on remission rates (Table 3.8). Five compared DMARD monotherapy with combination treatment: 3 of which used anti-TNF (Adalimumab) with Methotrexate (Soubrier et al. 2009, Kavanaugh et al. 2013, Detert et al. 2013) and 2 used combination DMARDs (Kawai et al. 2011, Montecucco et al. 2012). One trial compared combination DMARDs vs anti-TNF and Methotrexate (Moreland et al. 2012). All 6 RCTs reported DAS28 remission (Table 3.8-3.9). One trial reported DAS28CRP rather than DAS28ESR remission and the cut-off was unconventional at <2.6. 2 studies also report SDAI remission (Kavanaugh et al. 2013, Montecucco et al. 2012). The DAS28 remission rates for the monotherapy arms ranged from 17% - 39% and for the combination arms ranged from 34% - 59%. The SDAI remission rates were lower at 10%-16% and 20%-30.8% for monotherapy and combination arms respectively. The RCT comparing 2 different combination regimes reported similar remission rates in both arms (57% to 59%). From all 6 RCTs, in total: 142/674 (21%) patients achieved DAS28 remission with monotherapies and 498/1098 (45%) with combination therapies. This was similar to the original systematic review. No RCTs reported on ACR/EULAR Boolean remission.

**Table 3-8 Summary of Inclusion Criteria of Clinical Trials
(2009-2013), * = DAS28CRP, Results are mean values. RF = rheumatoid factor**

Study	Year	Jadad Score	Control					Treatment				
			Age (years)	Female (%)	RF+ve (%)	ESR	DAS28	Age (years)	Female (%)	RF+ve (%)	ESR	DAS28
Monotherapy vs Combination Therapy												
Soubrier et al.,	2009	2	49	81	77	35	6.15	46	78	70	39	6.13
Kawai et al et al	2011	3	50	81	-	46	-	47	90	-	45	-
Montecucco et al.,	2012	3	62	63	-	23.5	5.20	57	65	-	28	5.00
Detert et al.,	2013	4	53	67	59	36	6.3	47	61	55	33	6.2
Kavanaugh et al.,	2013	4	50	74	89		6.00*	51	74	87		6.00*
Combination vs Combination therapy												
Moreland et al.,	2012	2	49	77	92	33	5.8	51	74	89	37	5.8

Table 3-9 Remission Rates in Clinical Trials (2009-2013)

Study	Year	Disease duration (mths)	Follow-up (mths)	Remission	[§] Cases	Control		[§] Cases	Treatment	
						Treatment	Remission		Treatment	Remission
Monotherapy vs Combination therapy										
Soubrier et al	2009	6	12	DAS28ESR	29	MTX	11 (39%)	28	MTX/ adalimumab	17 (59%)
Kawai et al	2011	36	12	DAS28ESR	39	DMARDs	11 (28%)	56	Tacrolimus and DMARDs	27 (48%)
Montecucco et al	2012	12	12	1) DAS28ESR 2) SADI	90	MTX	1) 25 (27.8%) 2) 14 (16%)	96	MTX/pred	1) 43 (44.8%) 2) 30 (30.8%)
Detert et al	2013	12	6	DAS28ESR	57	MTX	17 (30%)	76	MTX/ adalimumab	36 (48%)
Kavanaugh et al	2013	12	6	1) DAS28CRP <2.6 2) SDAI	460	MTX	1) 78 (17%) 2) 46 (10%)	466	MTX/ adalimumab	1) 158 (34%) 2) 93(20%)
Combination vs Combination therapy										
Moreland et al	2012	36	24	DAS28ESR	132	MTX/HCQ/ SSZ	78 (59%)*	244	Etanercept/ MTX	139 (57%)*

§= Cases at end of follow-up, * = Achieving remission at some point during follow-up, MTX = Methotrexate, Pred = Prednisolone, DMARDs = disease modifying anti-rheumatic drugs

Only 2 RCTs reported radiographic outcomes (Table 3.10). Montecucco et al (Montecucco et al. 2012) showed that there were significantly more clinical remission (DAS28 <2.6) and ultrasound remission (PD negativity) with combination therapy than monotherapy. The OPTIMA study showed similar rates of SDAI remission in the non-radiographic progression group suggesting that aiming of a stringent target is more important than regimes used (Kavanaugh et al. 2013).

3.4.5 Combining the addendum RCTs to the original meta-analysis

5 RCTs compared remission rates between monotherapy and combination therapy arms. All of the new studies reported DAS remission. When these were combined with the original RCTs, the meta-analysis (Figure 3.3) showed the random effects OR for remissions with combination therapies compared with monotherapies was 2.15 (95% CI 1.75, 2.63). There was no evidence of significant heterogeneity. This result was very similar to the original meta-analysis.

Table 3-10 Summary of Radiographic Outcomes in RCTs

§ Combined clinical and ultrasound remission = DAS28 <2.6 and PD negativity

* = p <0.05 between treatment arms

Study	Year	Monotherapy	Combination Therapy
Montecucco et al	2012	PD negativity: 53.3% (48/90)* Combined clinical and ultrasound remission§: 15.8% (14/90)*	PD negativity: 69.8% (67/96)* Combined clinical and ultrasound remission§: 35.5% (48/96)*
Kavanaugh et al	2013	No radiographic progression at 26 weeks 1) DAS28CRP remission: 71% (17/24) 2) SDAI remission: 93% (28/30)	No radiographic progression at 26 weeks 1) DAS28CRP remission: 83% (52/63) 2) SDAI remission: 96% (54/56)

Review: remission
 Comparison: 01 Remission
 Outcome: 01 DAS Remission

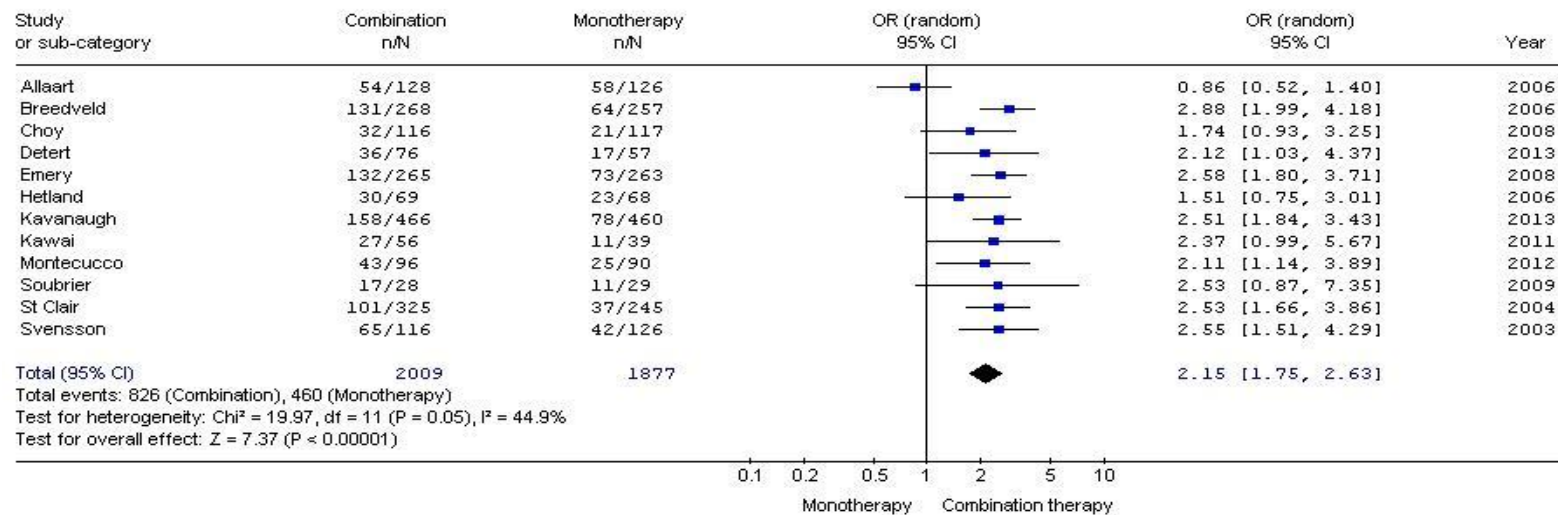


Figure 3-3 Forrest plot showing the meta-analysis of combined RCTs
 Using DAS remission criteria

3.5 Discussion

This systematic review showed that remission is becoming a realistic therapeutic target in early RA. Observational studies showed an overall remission rate of 17% with ACR remission criteria and 33% with DAS remission criteria. Many patients in clinical remission showed ongoing radiological progression. The RCTs showed that more patients achieved remission when combination treatments were used (random OR 1.69–2.01 compared to DMARD monotherapies). Radiological progression was less in patients receiving combination therapies who were in remission. The newer studies in the addendum showed similar results.

The ACR remission criteria and DAS28 remission criteria were derived using different methods, leading to differences in their definitions. Clinicians need to either agree on one measure of remission or, if agreement proves impractical, report both. One crucial difference between these criteria is the reliance placed on fatigue by the ACR criteria. Wolfe and colleagues highlighted the disproportionate impact of fibromyalgic rheumatoid on fatigue despite patients with this subtype having no more synovial inflammation (Wolfe, Hawley & Wilson 1996). Consequently, using fatigue to assess RA remission may disproportionately affect the assessment of fibromyalgic RA. Pain and fatigue are common in the general population and Sokka and colleagues suggested most people aged over 50 years in the general population will not fulfil ACR remission criteria for RA due to these symptoms (Sokka et al. 2007). The majority of the trials in this systematic review that used ACR remission criteria excluded fatigue.

Despite this, the ACR remission criteria are more stringent than DAS28-based criteria as no swollen nor tender joints are permitted in ACR remission criteria.

With DAS-based criteria, there is uncertainty about differentiating remission from low disease activity. The DAS-based remission criteria are derived from studies that showed that $DAS \leq 1.6$ best reflects ACR remissions (Prevoo et al. 1996). However, its conversion into $DAS28 \leq 2.6$ is controversial (Landewe et al. 2006); other levels of DAS28 have been suggested to better reflect remission (Makinen et al. 2005b, Fransen, Creemers & Van Riel 2004). Conversely, patients in remission may have falsely higher scores due to fibromyalgia or co-morbidities which can affect ESR, tender joint scores and patient global scores. DAS is not the only continuous assessment of disease activity and remission; other more stringent examples include the simple disease activity index (SDAI) and the clinical disease activity index (CDAI). Current cut points for remission have been defined as 3.3 for SDAI and as 2.8 for CDAI (Aletaha, Smolen 2005, Aletaha et al. 2005b). The SDAI has been reported in some of newer studies in the addendum. A final issue is the value of repeated assessments for determining remission; it is uncertain how many times patients need to be assessed and over what period; for instance, is remission on a single occasion important or does it need to be sustained for 6 or 12 months?

The relationship between disease activity and radiographic progression in early rheumatoid arthritis remains a topic of debate. The follow-up study of Cohen et al., found that sustained clinical remission correlated with stability of radiological damage in most patients (Cohen et al. 2007a). However, there was radiological

progression in a proportion of patients (16.7 %) in sustained remission and 20% developed erosions in a previously unaffected joint between the third and fifth years. Other trials have also reported radiological progression in patients in remission (Molenaar et al. 2004, Brown et al. 2008). It is therefore uncertain whether radiographic progression is wholly dependent on joint inflammation (Boers 2008). Another explanation may be that current assessment tools for disease activity are insensitive at low levels of inflammation and fail to detect ongoing disease activity. As a key goal of treatment is to prevent joint damage, radiological remission should be considered as a criterion for remission. The effect of treatment on radiological outcomes in patients with remission is unclear. The meta-analysis identified 2 RCTs, which reported radiographic outcomes in remission groups. They both found that combination therapy is associated with less radiographic progression in patients in remission when compared to monotherapy (Allaart, Breedveld & Dijkmans 2007, Svensson et al. 2005). Prednisone or anti-TNF were used in the combination arms of those trials in which there was reduced radiological progression. It is inappropriate to extrapolate results from these two trials to all combination DMARD regimens. Interestingly, a recent post-hoc analysis of the PREMIER study found that once patients are in sustained remission, there was no difference in radiographic progression across the treatment groups (Aletaha et al. 2009).

To conclude, remission is now a realistic treatment goal in early RA, particularly with the increased focus on patients receiving intensive combination treatment regimens. Currently, multiple remission criteria exist but DAS28 remission criteria appears easier to achieve. The absence of a single standard for assessing clinical remission is a

major hurdle in its use as a standardised outcome measure. In addition, radiological remission is currently not considered routinely in clinical trials, which is key to preventing long-term disability. Patients in true remission should be in clinical as well as radiological remission. Currently, there is an urgent need for international consensus on assessing and reporting true remission states. This will be further explored in chapters 5-7.

4 Predictors of Remission at 24 months

4.1 Introduction

Chapter 3 demonstrated that remission, particularly with the use of combination therapies, is now becoming a possibility in the treatment with RA. However, RA remains a heterogeneous disease with variable long-term outcomes that are difficult to predict. Its course ranges from drug free remission to severe joint damage and extra-articular manifestations (Scott, Steer 2007). Early intensive treatment has improved both clinical and radiological outcomes (Donahue et al. 2008, Ma, Kingsley & Scott 2010, Katchamart et al. 2008). This approach is now widely adopted as first-line treatment in routine clinical practice both nationally and internationally (NICE 2009, Singh et al. 2012, Smolen et al. 2010). Optimising therapeutic strategies to induce remission requires an understanding of the initial clinical characteristics that predict remission; currently no suitable model exists.

In the era of personalised medicine where treatment should be more individualised, it is unclear from the current literature whether all RA patients benefit from such intensive therapies to the same extent. Serological biomarkers including rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPA) play an important role in the diagnosis of RA (Aletaha et al. 2010). The presence of these antibodies are associated with radiographic damage, high disease activity and extra-articular manifestations (Nell et al. 2005, Smolen et al. 2006, van der Helm-van Mil et al. 2005).

There is emerging evidence that serological status can predict treatment response in biological therapies (Klaasen et al. 2011, Braun-Moscovici et al. 2006).

Accordingly, I set out to:

1) To develop a predictive model for 24-month remission in early RA patients. Data from an RCT comparing Methotrexate, steroids and combination DMARDs will be used to develop this model (CARDERA study)(Choy et al. 2008a). The predictive model will then be validated using data from a UK cohort - the Early RA Network (ERAN, (Kiely et al. 2009). These datasets provide a unique comparative assessment of remission in both a clinical trial and routine practice settings in patients seen across a large number of UK specialist centres.

2) To explore the use of the above model and serological biomarkers as predictors of response to DMARD intensive treatment. Since serum samples are only available in the CARDERA RCT, this part of the study is limited to patients enrolled in the RCT only.

4.2 Patients and Methods

This study was a post-hoc analysis of already collected data (CARDERA and ERAN cohorts). I carried out the autoantibody assays myself.

4.2.1 Clinical Trial Patients

The CARDERA trial recruited patients with RA from across 42 specialist rheumatology centres in England and Wales with less than 2 years disease duration who had

evidence of clinically active disease (Choy et al. 2008a). 467 patients were recruited and 378 patients remained on trial treatment and had full datasets at 24 months. 358 of these patients had available baseline serum samples. Patients were assessed initially and then 6 monthly for 24 months. The trial compared four treatment regimens, which were allocated randomly to across 4 equal treatment arms. These regimens comprised Methotrexate monotherapy, Methotrexate and Ciclosporin, Methotrexate and prednisolone, and Methotrexate, Ciclosporin and prednisolone. Methotrexate dose was 7.5-15mg, Ciclosporin dose was 100mg escalating to 3mg/kg and Prednisolone was 60mg aimed to stop by 34 weeks. For the purposes of this study, the double therapies were combined for analysis.

4.2.2 Observational Cohort Patients

The Early RA Network (ERAN) is an inception cohort of patients with newly diagnosed RA recruited in 19 UK centres. It reflected contemporary routine care of early RA patients (Kiely et al. 2009). We analysed data collected from 2002-2007. The 194 patients who completed 24 months follow-up at the time of analysis were evaluated. Patients were assessed at first presentation, at 3-6 months, and at 12 and 24 months. Treatment was determined by the supervising clinician. 94% of patients received initial DMARD monotherapy, comprising Methotrexate (47%), Sulfasalazine (42%), Hydroxychloroquine (7%), Leflunomide (2%), gold injections (1%) and Ciclosporin (0.5%). 4% of patients received two DMARDs initially and 2% started initial triple DMARD therapy. Oral or intramuscular steroids were used in 70% of patients.

4.2.3 Clinical Variables

Remission was defined as DAS28 scores of less than 2.60 (Prevoo et al. 1995). The following initial variables were present in both the clinical trial and the observational study: age, gender, rheumatoid factor positivity, rheumatoid nodules, health assessment questionnaire (HAQ) scores, tender joint counts for 28 joints (TJC), swollen joint count for 28 joints (SJC), erythrocyte sedimentation rate (ESR) and patient global assessments (PGA). Initial Larsen scores (as an assessment of radiographic progression) and SF36 scores were also available in the clinical trial.

4.2.4 Autoantibody analysis

Serum samples were taken at baseline and stored at -20°C prior to analysis. The autoantibody assays have been described in Chapter 2.

4.2.5 Statistical Analysis

Data were analysed using STATA version 10 (StataCorp, Texas, 2007) and IBM SPSS 20. Individual variables were assessed descriptively as median values and interquartile ranges. Categorical data were represented as percentages (%) and analysed using Chi-squared test if the $n \geq 10$ patients or Fisher's exact test if $n \leq 10$.

In assessing predictors of remission, analyses were restricted to those individuals with complete data at 24 months ($n=378$). Point remission at 24 months was used as the outcome as this was the study endpoint when the patients would have had maximal treatment. Univariate and multivariable logistic regression models were

used to estimate the associations between baseline variables that are potential predictors of remission at 24 months. In this analysis, variances were adjusted for inter-site effect using Huber-White sandwich (robust) estimator. All continuous measures were entered into the models in this format. The results were presented as univariate and multivariate odds ratios (OR) with 95% confidence interval, p-values were two-tailed throughout. Those variables that had p values ≤ 0.05 in the univariate analysis were carried forward into multivariate analysis. We also assessed whether variables were colinear for the final model; we considered variables were correlated if their correlation coefficient was >0.5 . The regression coefficient (β) of the variables from the final model was used to estimate the score. The predictive model was calculated to be: $(\beta_1 \times V_1) + (\beta_2 \times V_2) + (\beta_3 \times V_3) \dots + \alpha + e$, where V = independent variables and α = constant, e = error. This was similar to the method which was used to derive the prediction model for patients with undifferentiated arthritis who then develops rheumatoid arthritis, published by van der Helm-van Mil (van der Helm-van Mil et al. 2007). We also derived a simplified remission score, based on the factors identified in the regression analysis; details are given in the results section. The area under the curve from Receiver Operating Characteristic (ROC) was used to assess the model's ability to discriminate between remission and non-remission patients.

In assessing predictors of response to intensive therapy, analyses were restricted to the 358 individuals with complete data at 24 months and available serum samples. Gender, age and tender joint count were dichotomised: Male/Female, age under 50/over 50, TJC: 5 or less / 6 or over. The cut-off for TJC 5 or less/ 6 or over was chosen as this is considered the threshold for active disease in clinical trials. Logistic

regression models were used to estimate the associations between treatment regimens and point remission at 24 months when stratified by these clinical predictors and serological biomarkers. The effects of treatment on remission rates were first explored. This showed no difference between double vs monotherapy (OR 0.852 95% CI 0.435 – 1.67, $p = \text{ns}$). The effect of triple therapy compared to monotherapy was OR 2.22 95% CI 1.11-4.46 ($p = 0.025$). The models were therefore restricted to monotherapy vs triple therapy with adjustment for treatment centre. To explore the interaction between clinical and serological status, serological status models were also adjusted for baseline DAS28, gender and age. Multiple testing was adjusted by using bonferroni method.

4.3 Results

4.3.1 Patients and Remission Rates

The baseline characteristics of the 378 patients with full datasets were: 259 (68%) were females; median age was 54 years (IQR 46, 64); 82 (22%) had rheumatoid nodules. 74 (20%) were in remission at 6 months, 15% were in remission at 12 months and 21% at 24 months. The number of patients who did not achieve remission at any point during the study was 65%. 14% achieved remission at one time-point, 8% at 2 time-points and 7% had sustained remission over all time points. 9% achieved sustained remission between 12 and 24 months.

The observational cohort had enrolled 194 patients who had completed 24 months follow-up. They comprised of 140 (72%) females, their median age was 56 years (IQR: 47,66 years); 72% were rheumatoid factor positive and 28% had nodules. 24% achieved remission at 6 months, 20% at 12 months and 30% patients achieved remission at 24 months. 23% achieved remission at one time-point, 12% at 2 time-points and 4% had sustained remission over all time-points. The baseline characteristics of these 2 cohorts were similar with the exception of the baseline disease activity, which was expectedly higher in the RCT. The demographics of both patient populations are summarized in table 4.1.

Characteristics	Full dataset at 2 year	
	<i>Clinical Trial N=378</i>	<i>Observational Cohort N=194</i>
Female n (%)	259 (68)	140 (72)
Median Disease Duration (IQR) in months	2 (0, 5)	2 (1, 2)
Median Age at onset (IQR) in years	56 (46, 64)	55 (47, 64)
Clinical Rheumatoid Factor (%)	259 (68)	134 (72)
Rheumatoid Nodules (%)	82 (22)	54 (28)
Median Baseline DAS28 (IQR)	5.78 (4.88, 6.77)	4.77 (3.75, 5.77)
Median Baseline HAQ (IQR)	1.16 (0.12, 2.12)	1.25 (0.59, 1.75)
Median Larsen Score (IQR)	6.5 (2.5, 16.5)	-
Erosions at baseline (%)	-	56 (29)

Table 4-1: Patient Characteristics (CARDERA and ERAN)
Summary of the Clinical Trial (CARDERA) And Observational Cohort (ERAN)

4.3.2 Predictors of Remission at 24 months

4.3.2.1 Multivariate Remission Score

Regression Analysis Of Clinical Trial Data

Univariate analysis showed significant associations ($p < 0.05$) between remission at 24 months and age, gender, baseline HAQ, 28 tender joint count (TJC28), 28 swollen joint count (SJC28) and patient global scores. Multivariate logistic regression analyses showed only age, gender and TJC28 remained independently associated with remission at 24 months. Clinical rheumatoid factor, nodules, and baseline Larsen score did not affect remission (Table 4.2). The final model was assessed for correlations between variables to exclude colinearity; all the correlation coefficient was less than 0.5.

Developing Multivariate Remission Score Using Clinical Trial Data

A Multivariate Remission Score was generated using the coefficients from multivariable logistic regression analysis (Table 4.2). The Remission Score was $= 0.37 + [-0.03 \times \text{age}] + [1.1 \times \text{gender (1 for males and 0 otherwise)}] + [-0.07 \times \text{Baseline 28TJC}]$. A higher value indicates a higher probability that the patient will achieve in remission at 24 months. Remission Scores were calculated for each patient with a full dataset in the clinical trial. The area under the ROC curve was 0.71 (95% CI 0.63-0.77, Figure 4.1). When the "cut point" for the probability of identifying those patients in remission was set at 50% or more, the positive predictive value of the model was 69% and the negative predictive value was 81%.

Table 4-2: Predictors of Remission at 24 Months

Baseline Variables Predictive Of Remission At 24 Months In Clinical Trial (CARDERA) Using Univariate And Multivariate Logistic Regression

	Univariate		Multivariate		
	<i>OR (95%CI)</i>	<i>P-value</i>	<i>OR (95%CI)</i>	<i>P-value</i>	<i>Co-efficient (β)</i>
Age	0.98(0.96,1.00)	0.049	0.97(0.95,0.99)	0.014	-0.03
Male	2.91(1.75,4.85)	<0.001	3.14(1.80,5.46)	<0.001	1.1
Rheumatoid Factor (Clinical)	0.96(0.57,1.62)	0.884	-	-	-
Nodules	0.64(0.34,1.24)	0.186	-	-	-
Health Assessment Questionnaire	0.67(0.47,0.96)	0.027	1.07(0.66,1.72)	0.79	-
Larsen score	0.98(0.96,1.00)	0.057	-	-	-
Tender Joint Count	0.93(0.90,0.97)	<0.001	0.94(0.90,0.98)	0.006	-0.06
Swollen Joint Count	0.95(0.91,1.00)	0.03	1.00(0.95,1.05)	0.89	-
Erythrocyte Sedimentation Rate	0.99(0.98,1.00)	0.144	-	-	-
Patient Global Assessment	0.99(0.98,1.00)	0.041	0.99(0.98,1.00)	0.23	-
SF-36 Mental Component Score	1.02(1.00,1.03)	0.081			
SF-36 Physical Component Score	1.02(1.00,1.05)	0.097			

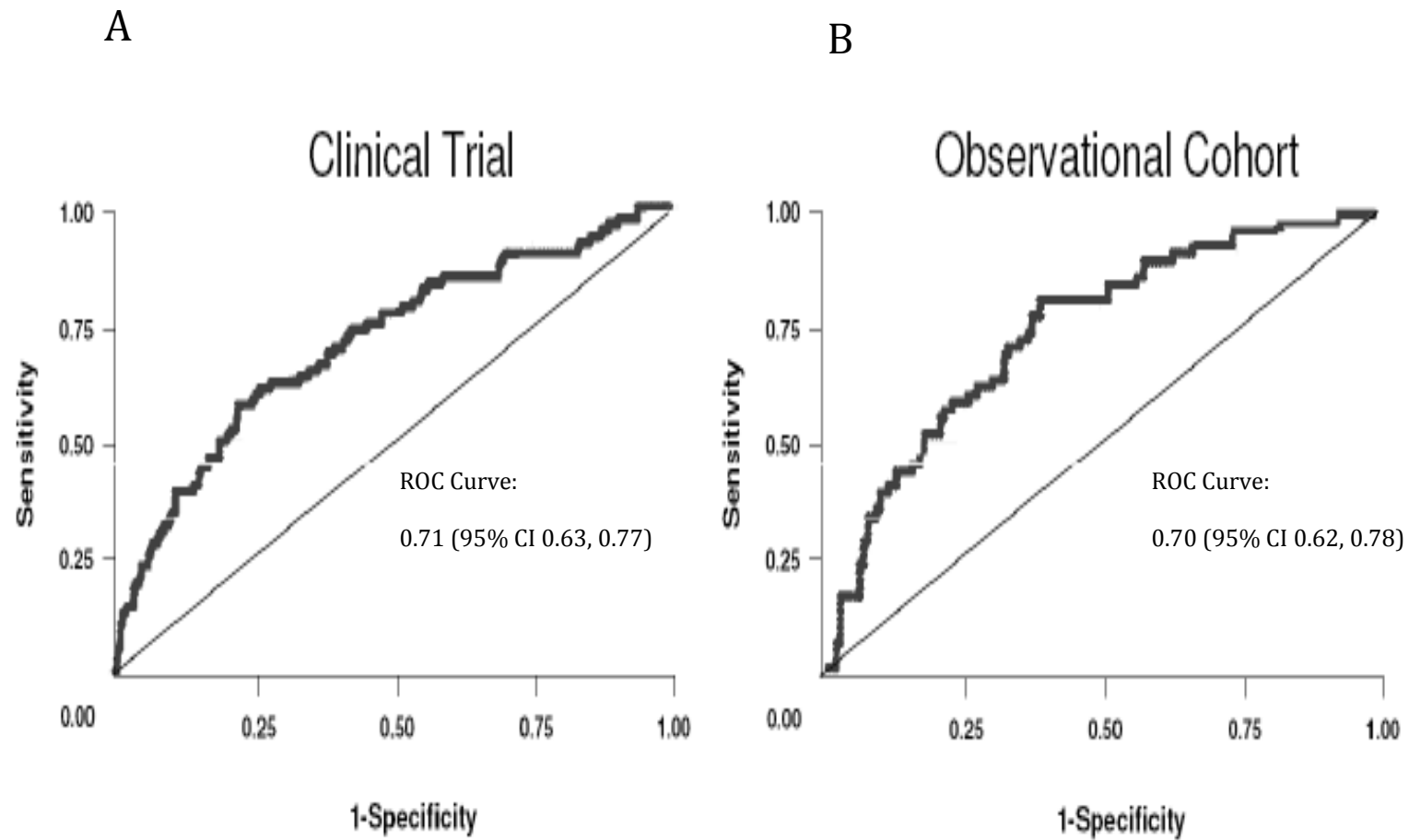
Replicating Multivariate Remission Score Using Observational Data

Applying the Multivariate Remission Score to patients in the observational study gave an area under the ROC curve of 0.70 (95% CI 0.62, 0.78 Figure 4-1). The model correctly classified 71% of patients with a poor sensitivity of 29% but good specificity of 90%. The positive predictive value was 53% and negative predictive value was 74%.

Predictive Model Using Multivariate Remission Score

The Multivariate Remission Score was applied to predict the likelihood that patients would achieve remission at 24 months in both CARDERA and ERAN (Figure 4-2). Using the Multivariate Remission Score, the more negative the scoring the higher the likelihood that the patient would not achieve remission at 24 months. Overall this approach correctly classified 80% of patients but had a poor sensitivity (13%) despite good specificity (98%). With low remission scores, patients were unlikely to achieve remission at 24 months.

Figure 4-1 ROC curves for The Remission Score
Receiver operating characteristics (ROC) curves using
(A) Clinical Trial dataset (CARDERA) And (B) Observational Cohort (ERAN)



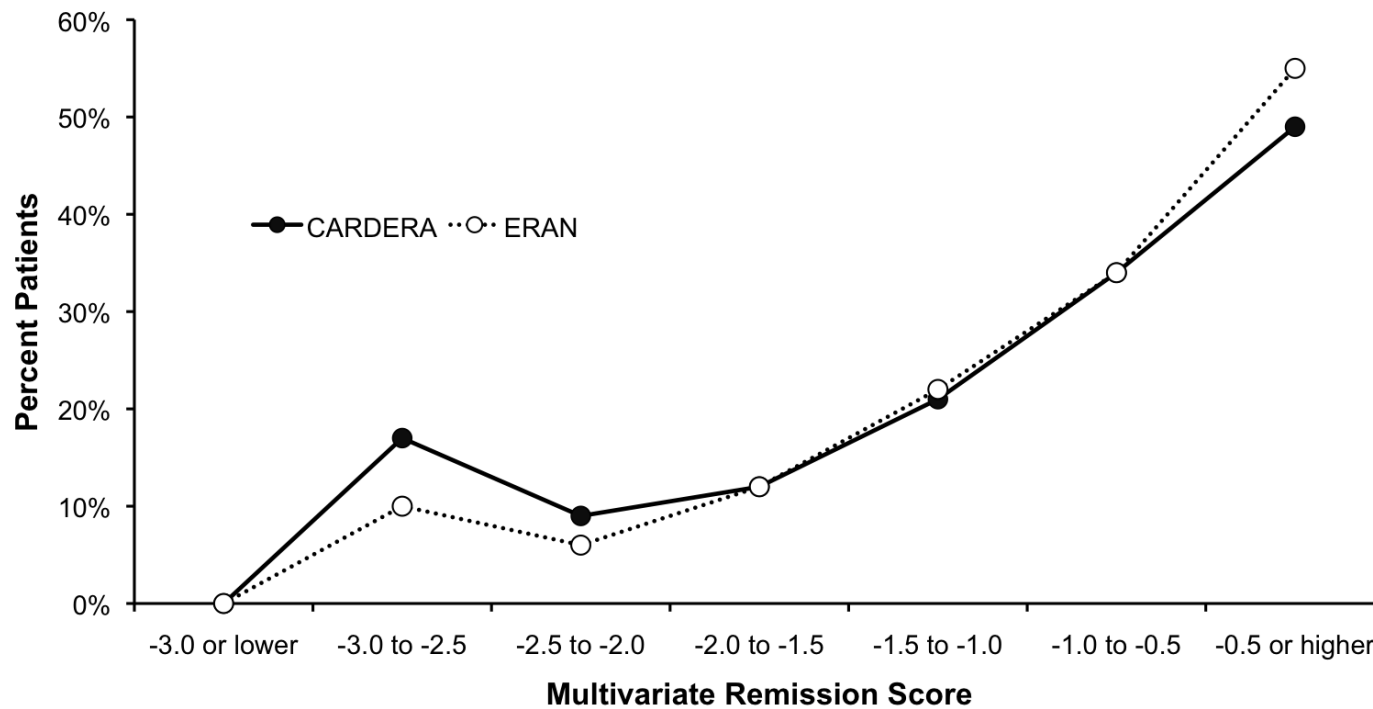


Figure 4-2. Predictive Ability of the “Multivariate Remission Score”

The Clinical Trial (CARDERA) And Observational Cohort (ERAN). This figure shows the % of patients who achieved remission at 24 months stratified according to the Multivariate remission score. The more negative the scoring the higher the likelihood that the patient would not achieve remission at 24 months.

4.3.2.2 Simplified Remission Score

As clinicians may not be keen to employ mathematical formulae when assessing patients, a simplified clinical tool was devised.

Firstly, patient divided by gender then by age (< 50 and > 50 years) and finally by baseline tender joint counts (<5, 6-19 and >20). The likelihood of achieving remission at 24 months was compared in the combined data from CARDERA and ERAN datasets. The results of this comparison are shown in Figure 4.3. Males aged under 50 years who had 5 or fewer tender joints when initially seen had the highest chances of achieving remission at 24 months (71% of patients). In contrast, females aged over 50 years who had more than 15 tender joints when first seen had the lowest chance of remission (11% of patients). Secondly, a Simplified Remission Score was devised by scoring 1 point for being male, 1 point for being aged < 50 years, 2 points for < or equal to TJC 5 and 1 point for 6-15 TJC. This simplified remission score showed a high correlation with the multivariate remission score (Spearman's correlation coefficient 0.80). For the purposes of comparison, we evaluated the likelihood of achieving DAS28 remission, low disease activity (DAS28 under 3.2) and having high disease activity (DAS28 5.1 or more). The results are shown in Figure 4.4. High Simplified Remission Scores (3 and 4), which were seen in 22% of patients, gave high chances of remission (37-72%). They also gave high chances of low disease activity (47-78%) and low chances of persisting high disease activity (12% or less). In contrast, low Simplified Remission Scores (0 and 1), which were seen in 41% of patients, gave low chances of remission (7-14%) and low chances of low disease activity (10-20%) and high chances of persisting high disease activity (34-67%).

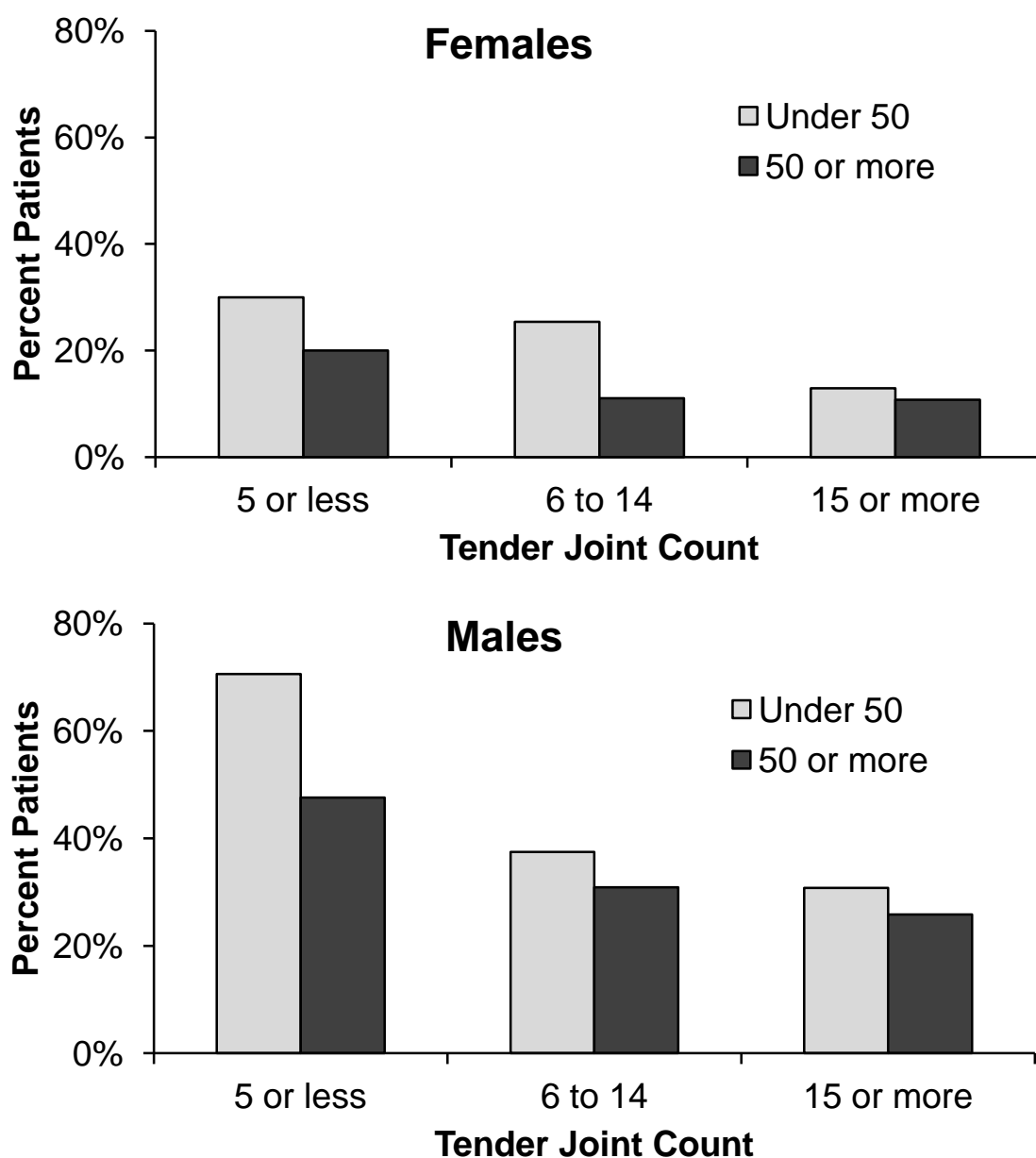


Figure 4-3. Relationship Of TJC28, Age And Remission According To Gender
The likelihood of achieving remission at 24 months was compared in the combined data from CARDERA and ERAN datasets. The patients were divided firstly by gender then by age and finally by baseline tender joint counts. The percentages of patients achieving remission at 24 months are shown in each of these subgroups.

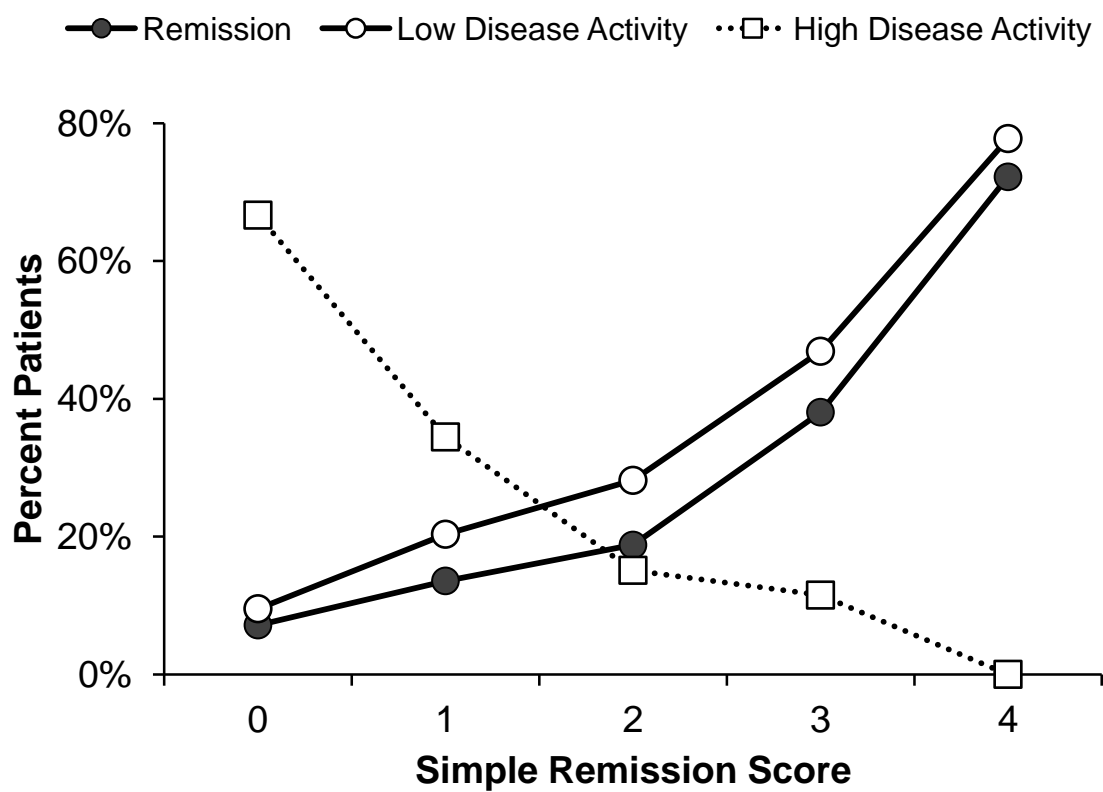


Figure 4-4: Simplified Remission Score and Disease Activity
The Ability Of "The Simplified Remission Scores" At Predicting Different Disease States At 24 Months In All Patients. Range 0-4

4.3.3 Predictors of response to treatment

4.3.3.1 Remission rates according to treatment groups

The followings section was based on post-hoc analysis of the CARDERA cohort only. The baseline characteristics of the CARDERA cohort with available serum are shown in Table 4.3. RF IgM and ACPA results were available for 358 and 351 patients. 89% were RF IgM positive and 73% were ACPA positive. These were representative of the total cohort. In total, 16/87 patients (18%), 29/180 (16%) and 30/90 (33%) patients achieved remission at 24 months using monotherapy, double therapy and triple therapy respectively.

Table 4-3 Baseline CARDERA Patient Characteristic with Available Serum
358 Patients had Complete 2 Year Data and Available Serum Samples. IQR = interquartile range, HAQ = Health assessment questionnaire, RF-IgM = Rheumatoid factor IgM isotype, ACPA = antibodies to citrullinated protein antigens

Clinical Features	Baseline Data
Female n (%)	245 (68%)
Median Age at onset (IQR)	54 (46, 63)
Rheumatoid Nodules n (%)	80 (22%)
Median Baseline DAS28 (IQR)	5.78 (4.88, 6.76)
Median Baseline HAQ (IQR)	1.62 (1.12, 2.03)
Median Larsen Score (IQR)	6.5 (2.3, 16)
RF-IgM positivity n (%)	313 (87%)
ACPA positivity n (%)	258 (72%)

4.3.3.2 The remission score and clinical predictors of remission by treatment group

The mean (SD) Remission Score was -1.7 (0.84). The Remission Score predicted treatment response in monotherapy, double and triple therapy (OR 3.07 95% CI 1.35-6.96 $p=0.007$, OR 1.99 95% CI 1.19, 3.32 $p = 0.008$ and OR 4.42 95% CI 1.90 – 8.94, $p < 0.0001$ respectively). This was adjusted for treatment centre.

The individual clinical predictors were then dichotomised: Gender – Male or Female, age - < 50 or ≥ 50 and TJC - < 6 or ≥ 6 . 245 patients were female, 113 patients were male, 122 were < 50 , 236 were ≥ 50 , 88 had less than 6 tender joints and 270 had 6 or more tender joints. Figure 4.5 shows treatment responses when stratified to different clinical predictors. Females achieved low levels of remission across all treatment arms and responded to a similar extent regardless of whether they received mono-, double or triple therapy [8/14 (14%), 17/131 13%, 13/57 23% respectively, $p > 0.05$]. Males responded better to triple therapy [17/33, 52%] compared to mono [8/31 26%, 12/49 25%]. Patients with lower TJCs responded to a similar extent across all the treatment groups: mono [6/19, 32%], double [12/44, 27%] and triple [10/24, 42%, $p = ns$] therapies. Patients with more than 6 TJCs achieved higher remission rates with triple therapy (20/66, 30%) when compared to mono (10/68 15%) and double (17/136 13%) therapies. Patients under 50 achieved similar high rates of remission across all the treatment groups: mono (11/32, 34%), double (14/61 23%) and triple (11/29 38%) $p = ns$ therapies. Patients over 50 years of age achieved higher remission rates using triple therapy (19/61, 31%) when compared to mono (5/55, 9%) and double (15/119, 13%) therapies.

Using logistic regression analysis, patients who were male, over 50 or had ≥ 6 TJC were more likely to achieve remission at 24 months using triple therapy compared to monotherapy (OR 2.99, 4.95 and 2.71 respectively, Table 4.4). There were no differences in response to monotherapy and triple therapy if patients were female, under 50 or had less than 6 tender joints (Table 4.4).

Table 4-4. Predictors of Remission When Stratified to Treatment Regimes
*Predictive Value Of Achieving Remission At 24 Months Using Triple Therapy
(Methotrexate, Ciclosporin and Prednisolone) When Compared To Methotrexate
Monotherapy Adjusted For Treatment Region*

Predictors Of Response	Odds Ratio	95% CI	P Value
Female	1.80	0.68 – 4.78	NS
Male	2.99	1.01 – 8.90	0.049
Over 50	4.95	1.66-14.75	0.004
Under 50	1.09	0.38 – 3.16	NS
≥ 6 TJC	1.56	0.43-5.63	0.028
<6 TJC	2.71	1.11-6.60	NS
RF-IgM Negative	1.49	0.17, 12.46	NS
RF-IgM Positive	2.28	1.08, 4.85	0.032
ACPA Negative	1.03	0.25, 4.30	NS
ACPA Positive	2.99	1.29, 6.97	0.011

4.3.3.3 Serological predictors of remission by treatment group

When stratified according to different treatment groups, serological status did have an impact on remission rates (Figure 4.5). In RF-IgM -ve patients, there was no difference in point remission rates between mono, double and triple therapies respectively [2/11 (18%), 5/23 (22%) and 3/10 (30%) $p > 0.05$]. In RF-IgM +ve patients, fewer patients achieved remission using monotherapy and double therapy (14/76, 18% and 24/157, 15%) when compared to triple therapy (27/80, 34%, $p = 0.02$). In ACPA -ve patients, 5/24 (21%), 4/42 (10%) and 5/22 (23%) achieved remission using mono, double and triple therapies respectively ($p > 0.05$). In ACPA +ve patients, more patients achieved remission using triple therapy (25/67, 37%) than monotherapy (11/63, 17%) and double therapy (24/132, 18%) ($p=0.007$).

The level of seropositivity was next explored. Patients were stratified into low-levels ($< 3 \times$ upper limit of normal) and high-levels ($\geq 3 \times$ upper limit of normal) of seropositivity as according to thresholds adopted in the ACR/EULAR criteria for Rheumatoid Arthritis in 2010 (12). In low-positive RF-IgM, there was no difference between remission rates in the different treatment groups: monotherapy 2/8 (25%), double therapy 0/15 (0%) and triple therapy 1/3 (33%, $p = ns$). In high-positive RF-IgM, more patients achieved remission with triple therapy 26/77 (33.8%) than monotherapy 12/68 (17.6%) and double 24/142 (16.9%, $p = 0.01$). In low-positive ACPA, there was no significant difference in remission rates between the treatment groups: monotherapy 3/5 (60%), double therapy 1/13 (7.7%) and triple therapy 2/9 (22%, $p = ns$). In contrast, in the high-positive ACPA group, more patients achieved remission with triple therapy 23/58 (39.7%) when

compared to monotherapy 23/76 (13.8%) and double 23/119 (19.3%, $p = 0.001$) groups.

The associations of treatment regimens and remission according to serological status are summarised in Table 4.4. The benefit of triple therapy is only apparent in RF IgM +ve (OR 2.28, 95% CI 1.08-4.85) and ACPA +ve (OR 2.99, 95% CI 1.29-6.97). Their effects size increased when adjusted for clinical factors (DAS28, age and gender) suggesting that the effects of the clinical and serological biomarkers were cumulative (OR 2.54 and 3.52 respectively Table 4.5).

Table 4-5: Serological Predictors Adjusted For Clinical Variable
*The Use Of Serological Status To Predict Remission At 24 Months
 Using Triple Therapy (Methotrexate, Ciclosporin and Prednisolone) Compared To
 Methotrexate Monotherapy. Adjusted For Treatment Region, Baseline DAS28, Gender
 And Age*

Predictors of response	OR	95% CI	P Value
RF-IgM Negative	1.17	0.58, 23.9	NS
RF-IgM Positive	2.54	1.12, 5.76	0.026
ACPA Negative	0.91	0.19, 4.28	NS
ACPA Positive	3.52	1.37, 9.03	0.009

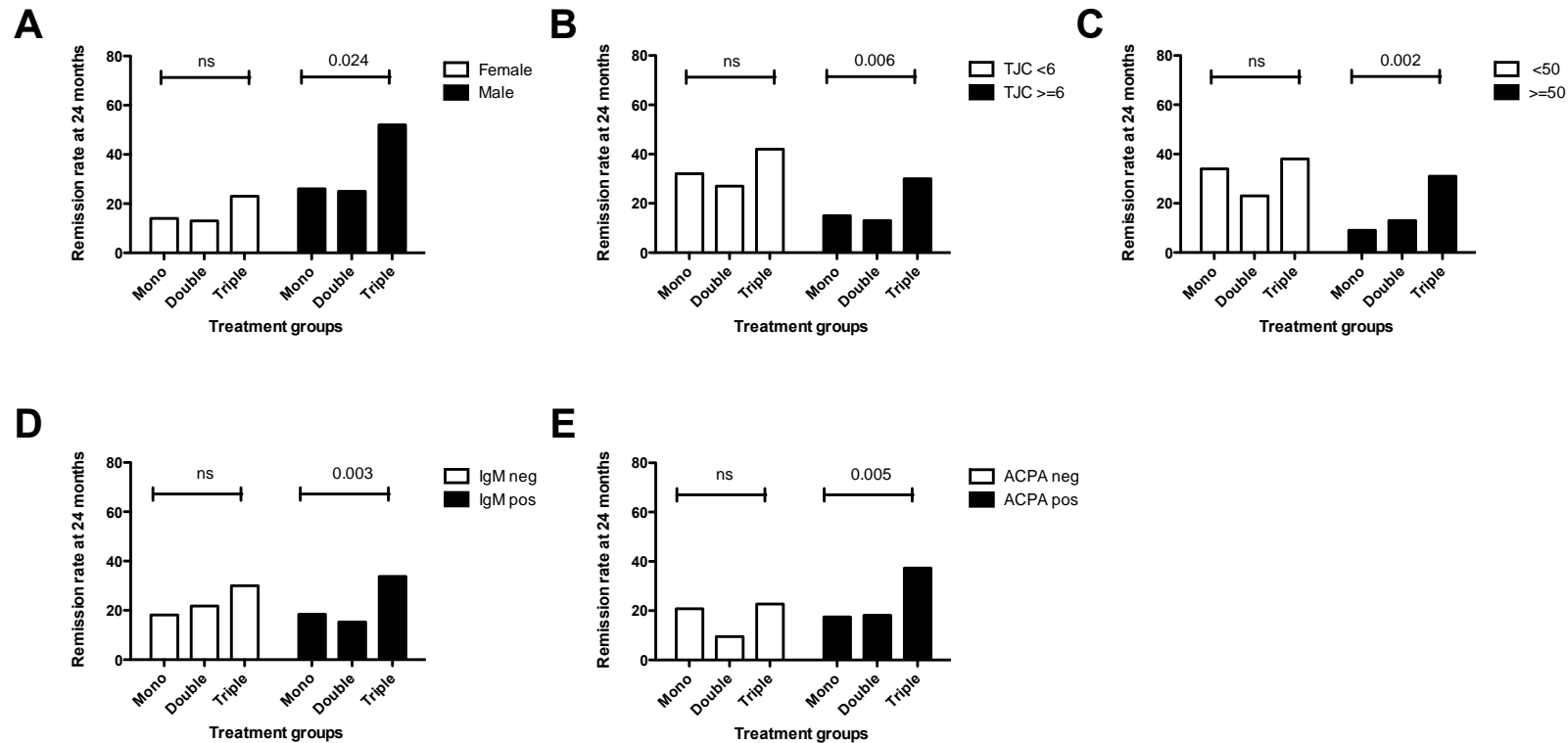


Figure 4-5: Effect of Clinical and Serological Predictors on Treatment Response

Showing Remission rates at 24 months in different treatment groups according to clinical and serological predictors.

(A) Gender, (B) Tender joint count, (C) Age, (D) RF-IgM and (E) ACPA. Multiple testing was adjusted by using bonferroni method. Monotherapy = Methotrexate, Double therapy = Methotrexate and Prednisolone or Methotrexate and Cyclosporin, Triple therapy = Methotrexate, Cyclosporin and Prednisolone

4.3.3.4 Serological Status And ACR Core Set Remission Measures

To explore the effects of the individual components of DAS28, the threshold levels for remission according to the ACR core set measures were used (12, 20). At 24 months, in total, 44.7% of patients achieved $\text{TJC28} \leq 1$, 22.9% had no swollen joints, 56.2% had $\text{ESR} \leq 20$ and 23.2% had $\text{PGA} \leq 10$. There were no differences between monotherapy and triple therapy in any of the 4 components at 24 months between RF-IgM positive and negative patients (Table 4.6). In ACPA +ve patients, more patients achieved TJC28 and SJC28 thresholds of remission in the triple therapy group than monotherapy groups at 24 months than ACPA negative patients (Table 4.6).

Table 4-6 Effects of Monotherapy and Triple Therapy on DAS28 Components

*Assessing the rate of remission at 24 months when stratified according to serological status within the individual DAS28 components
Tender joint count (TJC), Swollen Joint count (SJC), Erythrocyte sedimentation rate (ESR), Patient global assessment (PGA)*

Serological status	Treatment regimes	TJC28 at 24 months		SJC28 at 24 months		ESR at 24 months		PGA at 24 months	
		≤ 1	<i>p</i> value	< 1	<i>p</i> value	≤ 20	<i>p</i> value	≤ 10	<i>p</i> value
<i>RF IgM Negative</i>	Monotherapy	4/8 (50%)	ns	1/4 (25%)	ns	7/15 (47%)	ns	4/6 (67%)	ns
	Triple	4/8 (50%)		3/4 (75%)		8/15 (53%)		2/6 (33%)	
<i>RF IgM Positive</i>	Monotherapy	37/87 (43%)	ns	16/42 (38%)	ns	42/89 (47%)	ns	17/41 (42%)	ns
	Triple	50/87 (58%)		26/42 (62%)		47/89 (53%)		24/41 (59%)	
<i>ACPA Negative</i>	Monotherapy	14/25 (56%)	ns	5/10 (50%)	ns	12/27 (44%)	ns	7/13 (54%)	ns
	Triple	11/25 (44%)		5/10 (50%)		15/27 (56%)		6/13 (46%)	
<i>ACPA Positive</i>	Monotherapy	27/70 (39%)	0.015	12/36 (33%)	0.033	37/76 (49%)	ns	14/34 (41%)	ns
	Triple	43/70 (61%)		24/36 (67%)		39/76 (51%)		20/34 (59%)	

4.4 Discussion

In the first part of this study, we developed a remission score using simple clinical assessments that correctly classifies over 70% of patients. The score is equally applicable in a clinical trial and routine practice setting. Three variables – gender, age and initial tender joint counts – are all that is required. There was a marked difference in the likelihood of remission between different groups. Males who are under 50 years of age with less than 6 tender joints were the most likely to be in remission at 24 months. Clinical rheumatoid factor, rheumatoid nodules and x-ray damage did not contribute to the likelihood of remission. The Simplified Remission Score is readily applied in routine practice, and is similar in approach to the Simplified Disease Activity Index (Gaujoux-Viala et al. 2012). In the second part of this study, we explored these clinical factors as well as serological status in predicting treatment response to different treatment strategies. Our study suggests that triple therapy approach is only superior to monotherapy in certain subsets of patients. Stratifying patients according to gender, age, tender joints, RF isotypes positivity and ACPA positivity can predict response to intensive treatment.

The remission score is intended to identify those patients who are most likely to achieve remissions at 24 months using any treatment. We used similar prediction methods to those employed to predict the progression of RA from undifferentiated inflammatory arthritis (van der Helm-van Mil et al. 2007) and the likelihood of radiographic progression in RA (Vastesaeger et al. 2009). Our remission score was equally applicable in patients treated in a clinical trial and in routine practice, suggesting it is likely to be generalisable.

Gender, initial DAS28 scores and age have all been previously reported as important determinants of remission (Verstappen et al. 2005, Forslind et al. 2007, Schipper et al. 2010, Vazquez et al. 2007, Katchamart et al. 2010, Gossec et al. 2004). Interestingly, we found that it was tender joint counts rather than swollen joint counts or ESR values that were the defining clinical predictor of remission. The reason for this is uncertain. It might reflect greater levels of agreement between observers when assessing tender compared with swollen joint counts (Scott et al. 1996). Other possible explanations include the greater weighting of tender joint counts within the DAS28 score and the inter-relationship between tender joint counts and fibromyalgic RA (Leeb et al. 2004). Although we chose to use the individual components of the DAS28 rather than the DAS28 score itself to predict remission, using DAS28 instead of the tender joint count in the Multivariate Prediction Model gave a similar area under the ROC curve. Expectedly, patients with more tender joints (≥ 6) responded better to more intensive therapy. This study also showed that intensive therapy is not necessary in patients with milder disease as indicated by fewer tender joints as they responded with equal efficacy to monotherapy.

The impact of gender is so striking that treatments might need to be adjusted for gender; this is particularly relevant to the analysis of the outcomes of clinical trials where analyses should be separately carried out for different genders. Different risk stratifications by gender are recognised in a range of medical disorders, particularly in cardiovascular diseases (Lerner, Kannel 1986). The purpose of identifying patients' subsequent chances of remission when they are first seen is to

enable treatment to be personalised so that those patients with the least chance of remission can be targeted for more intensive treatment. However, given the findings from the second part of the study, females did not respond to a more intensive approach using triple therapy. Other approaches using different agents or tight-control regime should be considered.

The effect of age is more complex. The risk of remission was reduced in the elderly but they appear to respond to triple therapy better than monotherapy. However, in treating elderly patients more intensively, one should take into account of potential co-morbidities and adverse events (Wijnands et al. 1990) .

Intensive DMARD therapies are associated with increased drug toxicity (Ma, Cope & Scott 2010). Not all patients with RA require identical treatment. Instead, therapy should be individualized on the basis of risk factors assigned to each patient. A personalised, tailored approach to treatment, where each patient receives the appropriate intensity of treatment for as long as needed is the goal of treatment. This study suggests that males respond better to triple therapy compared to monotherapy whereas females respond equally but less well to all treatment regimes. Conversely, patients over 50 and with more than 6 TJC respond better to triple therapy than monotherapy but younger patients with a lower TJC respond well to all treatment regimes.

Several prediction matrices using serological status exist to predict risk of rapid radiological progression (RRP) using different DMARD and biological treatment regimes (Vastesaeger et al. 2009). Other studies have shown conflicting results

using serological status to predict anti-TNF response (Klaasen et al. 2011, Braun-Moscovici et al. 2006). However, no model exists for predicting clinical response to DMARD treatment regimes. This is the first study demonstrating the difference in response to intensive DMARD therapy between seronegative and seropositive RA patients.

In conclusion, remission in early RA can be predicted using three initial independent predictors – gender, age and tender joint counts. We created a multivariate remission score as well as a simplified remission score using these 3 predictors. These can be combined in a remission score which is best at predicting those patients who are unlikely to achieve remission at 24 months. This was validated in an independent observational cohort to improve the robustness of our study. It is premature to base current treatment on our remission score; further work is required to optimise this approach. However, the concept of a predictive remission score would be useful to tailor treatment regimes at an individual patient level. It is likely that a broader range of potential predictors need to be examined, particularly extended imaging and laboratory biomarkers, before such predictive assessment is adopted in routine practice. In addition, this study shows the importance of serological biomarkers to predict treatment response to combination DMARD therapy. ACPA in particular was shown to be the best biomarker for predicting treatment response. Although this is unlikely to be the only predictor of response, this study brings us a step closer to achieving personalised medicine in RA.

5 Clinical and radiological predictors of sustained remission

5.1 Introduction

The research in this chapter builds upon three themes. Firstly, the heterogeneous nature of RA outcomes; these were outlined in Chapter 1. Secondly, the increasing frequency of point remission states (Ma et al. 2010) which were considered in Chapter 3. Finally, the many different criteria used to define remission in patients with RA.

Many definitions of remission are based on continuous composite measures; one commonly used method is the Disease Activity Score (DAS, (Prevoo et al. 1996)) and its modifications, including DAS28-ESR (Fransen, Creemers & Van Riel 2004) and DAS28 CRP (Wells et al. 2009). Newer, stricter criteria are based on the Simplified Disease Activity Index (SDAI, (Aletaha, Smolen 2005) and Clinical Disease Activity Index (CDAI, (Aletaha et al. 2007)). The most stringent of remission criteria, the ACR/EULAR Boolean remission criteria (Felson et al. 2011), was designed to differentiate remission from low disease activity. All these criteria describe point remission (remission at one time-point) and none include the concept of time within their definitions.

The use of clinical and serological biomarkers as predictors of point remission at 24 months was evaluated in Chapter 4. The prediction model had good specificity but poor sensitivity. This suggests that other laboratory biomarkers are likely to be required. A more complete clinical picture of disease activity necessitates integrating data points over time. True remission should mean that patients are in

sustained remission over time. Sustained remission has also been shown to arrest radiographic progression irrespective of treatment used in the PREMIER study (Aletaha et al. 2009). Currently in the literature, sustained DAS28 remission is reported infrequently in studies. Moreover, sustained remission using the more contemporary remission criteria have been described in only 2 studies to date (Svensson et al. 2013, Prince et al. 2012).

The systematic review carried out in Chapter 3 showed that radiographic progression still occurred. Several studies have reported on the presence of subclinical synovitis in cohorts of remission patients (Ten Cate et al. 2000). Furthermore, the presence of power Doppler signal have been shown to be a powerful predictor of radiographic progression (Brown et al. 2008, Foltz et al. 2012) and flare (Saleem et al. 2012, Peluso et al. 2011, Scire et al. 2009) in remission states. Data on the ability of ultrasound to differentiate between LDAS and different remission states is conflicting. Balsa et al., 2010, reported superiority of SDAI over DAS28 remission criteria in the assessment of ultrasound-classified remission (Balsa et al. 2010). However, this was not confirmed by another study which showed similar PD signal between DAS28, SDAI and Boolean remission and low disease activity (Saleem et al. 2011).

To explore further whether laboratory or radiological biomarkers could be included to better characterise low disease activity states, and how these relate to longer term outcomes such as sustained remission or radiographic progression, a new cohort of RA patients was established with features of stable low disease activity states; the REMIRA cohort (REMission In RA). It had two main goals. Firstly, determining the prevalence of sustained remission over 1 year using the

different remission criteria. Secondly, assessing the value of combining clinical, serological and radiological biomarkers as predictors of sustained remission in patients who have already achieved low disease activity states.

5.2 Methods and Patients

5.2.1 REMIRA cohort

Adult RA patients diagnosed according to the 1987 revised ACR criteria were recruited into the REMIRA (REMission in RA) study. Inclusion criteria restricted disease duration to <10 years (as defined by date from diagnosis). We chose this as a cut-off as we were particularly interested in patients who were more likely to have had contemporary treatment of RA. To ensure all recruited study subjects were in stable LDA, we also included the following criteria: stable DMARDs therapy > 6 months and DAS28-ESR ≤ 3.2 for at least 1 month. Three centres across south London participated in this study: Guy's and St Thomas' Hospital, King's College Hospital and University Hospital Lewisham NHS Foundation Trusts. The study was approved by the local ethics committee and conducted according to the guidelines of the Declaration of Helsinki (REC:09/H0803/154, Wandsworth Research Ethics Committee). Local Research and Development (R&D) approval was obtained from all sites. Written informed consent was obtained from all participants.

5.2.2 Clinical assessments

Clinical data including demographics, smoking, disease duration and treatment were collected. For the evaluation of pain, fatigue, patients' as well as evaluators' global assessment of disease activity, 100mm visual analogue scales (VAS) were

used. Early morning stiffness (EMS) was recorded in minutes. Erythrocyte Sedimentation Rate (ESR) and C-reactive protein (CRP) were collected from patient records (within the month of clinical assessment). For the evaluation of remission, DAS28-ESR, DAS28-CRP, SDAI, CDAI and ACR/EULAR Boolean remission criteria were collected. Patients were assessed every 3 months for 1 year.

5.2.3 Autoantibody analysis

Serum samples at baseline were obtained for autoantibody testing. The commercial kits used to determine RF and anti-CCP status were the same as those described in chapter 4.

5.2.4 Radiographs of hands and feet

Posterior-anterior conventional radiographs of the hands and feet were taken at entry of study and 12 months. Erosive progression was defined as new or larger erosions over 1 year. This was scored by me as first reader and Prof David Scott as second reader. The second reader read a selection of 10% of radiographs.

5.2.5 Ultrasound assessments

Ultrasonography was carried out in all patients at baseline and 12 months. This has been described in Chapter 2.3.2.2. This was scored by me as first reader and Dr Toby Garrood as second reader. The second reader scored 20% of patients at one timepoint, selected at random, to assess inter-observer reliability. Intra-observer reliability was assessed by selecting the same images as the second reader. These

were read 6 months later. Scoring was carried out retrospectively on acquired images to ensure that both ultrasound scorers were blinded to clinical findings.

5.2.6 Statistical analysis

STATA 11.2 (StataCorp, College Station, TX, USA) was used for statistical analysis. Point remission at baseline was defined as DAS28-ESR <2.6 , DAS28-CRP <2.32 , SDAI ≤ 3.3 and CDAI ≤ 2.8 . ACR/EULAR Boolean remission was defined as TJC, SJC, CRP (mg/dl) and patient VAS (0-10cm) all ≤ 1 . Sustained remission (SR) was defined as achieving remission at all five study visits, intermittent remission (IR) as achieving remission on at least 1 study visit but not all. No remission (NR) was defined as not achieving remission at any study visit during the one year of follow up. Low disease activity (LDAS) was defined as no remission by any criteria and NR is defined as no remission at all time-points; for DAS28-ESR these would include study subjects whose disease activity remains in the > 2.6 to < 3.2 range. Individual variables were assessed descriptively as median values and interquartile ranges (IQR). Agreement between the different remission criteria was assessed using Cohen's kappa test. Intra- and inter-reader reliability for scoring ultrasound was assessed using Intraclass Correlation Coefficient (ICC). 0 is defined as 'non-existing', between 0 and 0.2 = 'slight', between 0.2 and 0.4 = 'fair', between 0.4 and 0.6 = 'moderate', between 0.6 and 0.8 = substantial, between 0.8 and 1.0 'almost perfect'. Categorical data were analysed using Chi-squared test.

Ethics approval was not in place at the start of the study to collect data on Euroqol (n=17) and FACIT-F (n=14). Therefore, T3 data were used as baseline data for these subjects. If there were missing data at baseline in the other parameters, the

mean values for the year for that particular patient were used. Last observation carried forward (LOCF) was used for missing data for follow-up visits.

Extended analyses were limited to DAS28-ESR, SDAI and Boolean remission. CDAI and DAS28CRP were excluded as their agreements were similar to SDAI and DAS28ESR respectively. Univariate logistic regression models was used to estimate the associations between variables that are potential predictors of sustained remission. The odds ratio (OR) and 95% confidence intervals (95% CI) represent the increased or decreased risk associated with a 1-unit change in the predictor variable for continuous variables. For dichotomous variables, the OR represents the risk associated with having the characteristic compared to the risk of not having it. With continuous variables, it can be difficult to interpret the actual differences amongst variables because variables can be scaled in different units. The standardized odds ratio, or the odds ratio per 1-SD change, allows comparison between predictor variables using common units. Standardised OR is defined as $\text{Exp}(B \times \text{SD})$ where B = B co-efficient and SD = standard deviation. Unfortunately, multivariate logistic regression analysis could not be carried due to the small numbers in each of the groups.

5.3 Results

5.3.1 Patient Cohort

372 patients were identified in LDAS or remission states. 222 patients did not fulfill criteria the inclusion criteria and 46 patients declined to participate. 104 patients were enrolled in the observational study: 4 dropped out; 100 patients completed 12 months follow up. The drop-outs were all due to patients' choice.

The baseline features of the patients enrolled in the study are summarised in Table 5.1. Their median age was 56 years and median disease duration was 45 months; 63% were female, 88% were IgM RF positive and 72% were ACPA positive. Median scores for DAS28-ESR, DAS28-CRP, SDAI and CDAI were all low, in the range 2.10 to 3.60. Most patients (87%) were receiving Methotrexate. Between 4% and 31% were receiving other DMARDs (sulphasalazine, Hydroxychloroquine and Leflunomide). Only 3% were being treated with prednisolone and 16% were treated with TNF inhibitors. 57% were on DMARD monotherapy, 36% on DMARD double therapy and 7% on DMARD triple therapy.

Table 5-1 Baseline Characteristics Of The REMIRA Cohort 9 (n = 104)

Baseline Characteristics		
Age, Median (IQR) years		56 (47-69)
Disease Duration In Months, Median (IQR)		45 (23-75)
Female, Percent		63%
Igm RF+Ve, Percent		88%
Anti-CCP +Ve, Percent		72%
<i>Ethnicity</i>	Caucasian	74%
	Asian	7%
	Afro-carribean	19%
TJC28, Median (IQR)		0 (0,1)
SJC28, Median (IQR)		0 (0,1)
ESR, Median (IQR)		8 (4.75 – 16)
Patient Global, Median (IQR) (0-100mm)		18 (10, 35)
DAS28ESR, Median (IQR)		2.10 (1.40-2.78)
DAS28ESR Remission, Percent		66
DAS28CRP, Median (IQR)		2.15 (1.79-2.72)
DAS28CRP Remission, Percent		59
SDAI, Median (IQR)		3.60 (1.70-7.56)
SDAI Remission, Percent		46
CDAI, Median (IQR)		3.20 (1.20 – 7.20)
CDAI Remission, Percent		46
Boolean Remission Criteria, Percent		30%
HAQ, Median (IQR)		0.125 (0, 0.75)
EQ5D, Median (IQR)		0.80 (0.69 – 0.88)
EQ5D VAS, Median (IQR)		80 (70-90)
FACIT-F, Median (IQR)		42 (35-47)
SF36 MCS, Median (IQR)		52 (44-58)
SF36 PCS, Median (IQR)		44 (38-52)
Erosive Disease, Percent		52%
Erosive Progression, Percent		14%
<i>Treatment</i>	Methotrexate	87%
	Sulphasalazine	27%
	Hydroxychloroquine	31%
	Leflunomide	4%
	Prednisolone	3%
	Combination therapy	43%
	TNF Inhibitors	16%

5.3.2 Baseline Remissions

At baseline 67/102 (66%) were in DAS28ESR remission and 35/102 (34%) were in LDAS states. In addition 58 (59%) were in DAS28CRP remission, 45 (46%) in SDAI remission, 47 (46%) in CDAI remission and 30 (30%) in Boolean remission. Comparing agreement between remission states using kappa statistics (Table 5.2) showed close agreement between Boolean, SDAI and CDAI remission criteria (84%-99%). By contrast DAS28ESR remissions had lower levels of agreement, particularly with Boolean remissions.

Table 5-2 Agreement Between Baseline Point Remission Criteria
Results Shown For Cohen's kappa

	Boolean	SDAI	CDAI	DAS28CRP
SDAI	85%			
CDAI	84%	99%		
DAS28CRP	70%	86%	87%	
DAS28ESR	60%	69%	69%	80%

5.3.3 Intermittent And Sustained Remissions

The frequencies of intermittent and sustained remissions are shown in Figure 5.1. Intermittent remissions occurred in 38% to 45% of patients, depending on the remission criteria used. Intermittent DAS28ESR remissions were seen in 44 (42%) patients, DAS28 CRP remissions in 43 (41%) patients, SDAI remissions in 47 (45%) patients, CDAI remissions in 46 (44%) patients and Boolean remissions in 39 (38%) patients.

Intermittent remissions occurred in 10% to 47% of patients, depending on the remission criteria used. Intermittent DAS28ESR remissions were seen in 49 (47%) patients, DAS28 CRP remissions in 34 (33%) patients, SDAI remissions in 23 (22%) patients, CDAI remissions in 24 (23%) patients and Boolean remissions in 10 (10%) patients. In addition 10 (10%) of patients were in no remission states. Comparing agreement between sustained remission states using kappa statistics (Table 5.3) showed close agreement between Boolean, SDAI and CDAI remission criteria (87%-99%) Once again sustained DAS28ESR remissions had lower levels of agreement, particularly with sustained Boolean remissions.

Table 5-3: Agreement Between Sustained Remission Criteria.
Results Shown For Cohen's kappa

	Boolean	SDAI	CDAI	DAS28CRP
SDAI	88%			
CDAI	87%	99%		
DAS28CRP	77%	89%	90%	
DAS28ESR	63%	71%	72%	80%

5.3.4 Remission And Disease Activity

The median DAS28 score over 12 months for the whole cohort was 2.06 (IQR 1.32, 2.71). The median DAS28 scores over 12 months for the no remission group was 3.75 (IQR 3.47), for DAS28ESR intermittent remission was 2.54 (2.29, 2.84), for DAS28ESR sustained remission was 1.31 (0.95, 1.76), for SDAI intermittent remission was 1.93 (1.35, 2.30), for SDAI sustained remission was 1.03 (0.67, 1.41), for Boolean intermittent remission was 1.61 (1.14, 2.07) and for the Boolean sustained remission was 0.72 (0.63, 1.30).

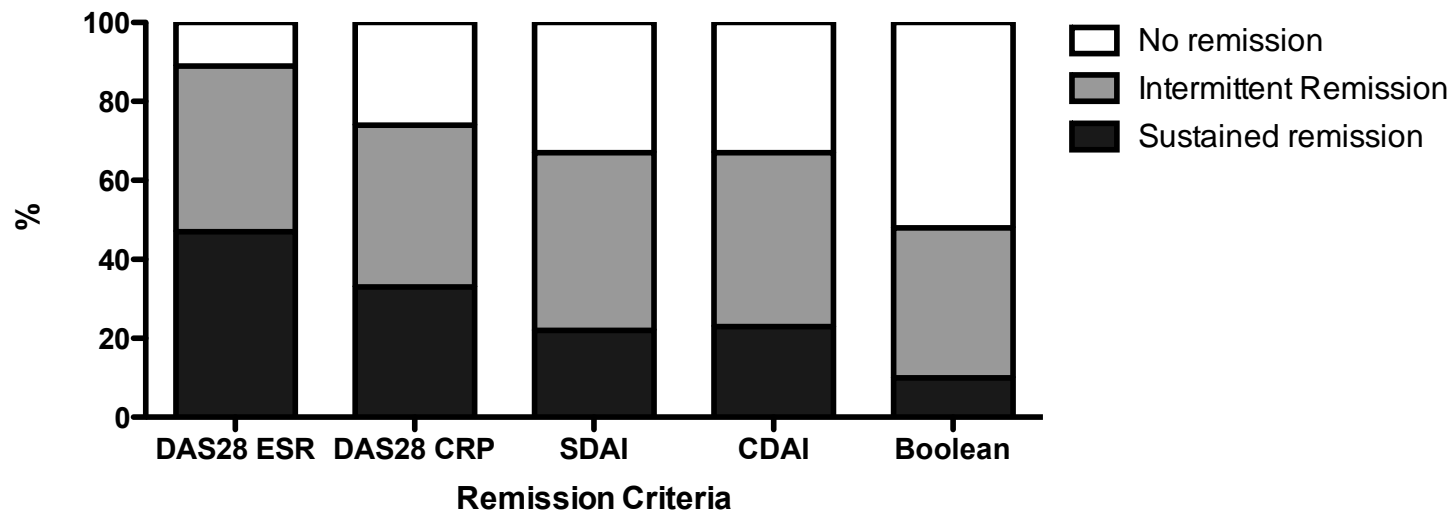


Figure 5-1: The Frequency of Remission in the REMIRA Cohort

The frequency of no remission, intermittent remission and sustained remission in the REMIRA cohort according to the different Remission criteria.

5.3.5 Radiographic Outcomes

Paired baseline and 12-month radiographs were available in 98 patients: 14 had erosive progression and developed new erosions or showed worsening of existing erosions. Seven of these 14 patients were in DAS28 sustained remission, 2 in SDAI sustained remission and 2 in Boolean sustained remission. There was no evidence of differences in radiographic progression between LDAS and remission groups.

5.3.6 Ultrasound Assessments

103 patients had baseline ultrasound images (SH and PD); 82 patients had 12-month SH images and 76 had 12 month PD images. Inter-observer reliability was good: ICC was 0.62 for SH and 0.98 for PD; intraobserver reliability was 0.79 for SH and 0.99 for PD. The ultrasound findings are summarized in Table 5.4. The median baseline SH, baseline PD, 12-month SH and PD were 11.7, 2.0, 11.5 and 0.7 respectively. The numbers (%) of patients without PD signal were 10 at baseline and 9 at 12 months. All patients in the cohort had evidence synovial hypertrophy at baseline and 12 months (ie SH score >1).

There were no differences between baseline SH and PD, no remission and any the remission groups (Table 5.5). No remission patients had more 12-months SH compared to remission groups (Table 5.5). There was a similar trend for 12-month PD, though this difference was not statistically significant (p values 0.051 – 0.074).

Table 5-4 Baseline And Follow Up Ultrasound Scores
SH = synovial hypertrophy, PD = power Doppler. Scores from 0 -36

	Median	IQR
<i>Baseline Total SH</i>	11.7	8 – 14.3
<i>Baseline Total PD</i>	2	1-4.3
<i>Twelve Month Total SH</i>	11.5	8.3 – 14.7
<i>Twelve Month Total PD</i>	0.67	1.33 – 3.67

Table 5-5 Ultrasound Summary In no Remission (NR) and sustained Remission Groups

Showing baseline and 12 Month synovial hypertrophy (SH) and power doppler (PD) scores in No Remission and sustained remission groups. Median Values (IQR) Shown, The level of significance is determined Mann-Whitney U test (LDAS vs each remission groups)

Ultrasound	No Remission	DAS28ESR Remission	P Value	SDAI Remission	P Value	Boolean Remission	P Value
<i>Baseline SH</i>	13.3 (10.0, 17.0)	11.5 (7.8, 15.0)	0.22	12.3 (8.0, 14.3)	0.32	8.7 (7.0, 12.0)	0.105
<i>Baseline PD</i>	2.8 (1.7, 7.3)	2.0 (1.0, 3.7)	0.18	1.7 (0.7, 4.7)	0.13	1.5 (1.0, 2.0)	0.075
<i>12 Month SH</i>	15.0 (12.5, 18.2)	10.3 (8.3, 14.7)	0.013	10.3 (8.0, 14.0)	0.005	9.3 (8.0-11.67)	0.004
<i>12 Month PD</i>	2.8 (1.2, 13.2)	1.0 (0.3, 3.3)	0.051	1.0 (0.3, 3.3)	0.051	1 (0.33 – 2.67)	0.074

5.3.7 Ultrasound Predictors Of Radiographic Progression

The role of ultrasound parameters as predictors of radiographic progression was next assessed (Table 5.6). Both SH and PD at baseline were found to be predictors of radiographic progression at 12 months (OR 1.15 and 1.58 respectively). At 12 months, only PD was a predictor (OR 1.10).

Table 5-6: Ultrasound Predictors of Radiographic Progression
Using Univariate logistic regression, PD = Power Doppler Signal, SH = Synovial Hypertrophy

Ultrasound Variables	Odds Ratio (95% CI)	P Value
<i>Total SH baseline</i>	1.15 (1.03, 1.29)	0.017
<i>Total SH 12 months</i>	1.06(0.96, 1.18)	0.255
<i>Total PD baseline</i>	1.58 (1.03, 2.42)	0.036
<i>Total PD 12 months</i>	1.10 (1.003, 1.20)	0.043

5.3.8 Baseline Clinical Predictors Of Sustained Remission

Logistic regression analysis showed predictors of sustained DAS28ESR remission at baseline included ethnicity (OR 0.06, 95% CI 0.12, 0.30), ESR (OR 0.66, 95% CI 0.51, 0.84), CRP (OR 0.49 95% CI 0.31, 0.78), TJC (OR 0.26, 95% CI 0.12, 0.55), patient global assessment (OR 0.90 95% CI 0.85, 0.96) and physician global assessment (OR 0.83 95% CI 0.75, 0.92). These findings are summarised in Table 5.7

The predictors of sustained SDAI remission are shown in Table 5.8. These included ethnicity (OR 0.10 95% CI 0.02 - 0.58), baseline ESR (OR 0.85 95% CI 0.77- 0.95), baseline CRP (OR 0.55 95% CI 0.33 - 0.90), baseline TJC (OR 0.07 95% CI 0.01 - 0.62), baseline patient global assessment (OR 0.82 95% CI 0.69 - 0.96), baseline physician global assessment (predicts perfectly). With regards to baseline physician global assessment, all patients in sustained SDAI remission had a score of 0 whereas the score in the NR group ranged from 5-30.

The predictors of sustained Boolean remission are shown in Table 5.9. These included erosive disease at baseline (OR 13.50 95% CI 1.20, 152.21) and patient global assessment (OR 0.84 95% CI 0.73, 0.98). Baseline ESR, TJC and physician global assessments predicted perfectly. In the sustained Boolean remission group, the baseline ESR range were between 0-8, TJC 0 and Physician physician global assessment 0 whereas in the NR group, the baseline ESR range were between 10-37, TJC1-3 and Physician physician global assessment 5-30.

Table 5-7 Clinical, Serological and Radiological Predictors of DAS28ESR SR

*Using univariate logistic regression, SR = sustained remission, *where Afrocarribean/Asian = 1, Caucasian = 0*

LDAS vs DAS28ESR SR	OR (95% CI)	Standardised OR	p value
ACPA positivity	0.88 (0.196, 3.94)	-	0.87
Afrocarribean/Asian vs Caucasian *	0.06 (0.12, 0.301)	-	0.001
Age	0.97 (0.92, 1.02)	0.65	0.256
Disease duration	1.00 (0.99, 1.01)	0.93	0.835
Erosive baseline	1.63 (0.41, 6.52)	-	0.489
Female	0.89 (0.22, 3.55)	-	0.868
Baseline ESR	0.66 (0.51, 0.84)	0.02	0.001
Baseline CRP	0.49 (0.31, 0.78)	0.05	0.003
Baseline TJC	0.26 (0.12, 0.55)	0.25	<0.0001
Baseline SJC	0.63 (0.30, 1.33)	0.46	0.224
Baseline PtGA	0.90 (0.85, 0.96)	0.15	0.001
Baseline PhGA	0.83 (0.75, 0.92)	0.09	<0.0001
RF IgA positivity	1.14 (0.25, 5.09)	-	0.571
RF IgM positivity	2.10 (0.19, 23.09)	-	0.096
Total PD baseline	0.88 (0.75, 1.02)	0.53	0.091
Total SH baseline	0.89 (0.75, 1.07)	0.59	0.227

Table 5-8 Clinical, Serological and Radiological Predictors of SDAI SR

Using univariate logistic regression, SR = sustained remission, *where Afrocarribean/Asian = 1, Caucasian = 0

** empty = one of the 4 groups on crosstab had no data.

LDAS vs SDAI SR	OR	Standardised OR	p value
ACPA positive	0.64 (0.13, 3.25)	-	0.593
Afrocarribean/Asian vs Caucasian *	0.10 (0.02, 0.58)	-	0.01
Age	0.98 (0.93, 1.04)	0.77	0.51
Disease duration	1 (1, 1)	0.92	0.82
Erosive baseline	2.33 (0.51, 10.64)	-	0.27
Erosive progression	1.22 (0.14, 10.95)	-	0.859
Female	1.25 (0.27, 5.77)	-	0.775
Baseline ESR	0.85 (0.77, 0.95)	0.25	0.004
Baseline CRP	0.55 (0.33, 0.90)	0.09	0.017
Baseline TJC	0.07 (0.01, 0.62)	0.07	0.017
Baseline SJC	0.26 (0.07, 0.89)	0.10	0.032
Baseline PtGA	0.82 (0.69, 0.96)	0.02	0.015
Baseline PhGA	Predicts perfectly		
RF IgA positivity	1.09 (0.22, 5.45)		0.916
RF IgG positivity	Empty** (,)	-	-
RF IgM positivity	3.14 (0.17, 57.08)	-	0.44
Total PD baseline	0.79 (0.58, 1.06)	0.30	0.112
Total SH baseline	0.89 (0.72, 1.10)	0.59	0.29

Table 5-9 Clinical, Serological and Radiological Predictors of Boolean SR

*Using univariate logistic regression, SR = sustained remission, *where Afrocarribean/Asian = 1, Caucasian = 0, **omitted = no sustained Boolean remission patients were IgG positive*

LDAS vs Boolean SR	OR	Standardised OR	p value
ACPA positive	0.11 (0.01, 1.34)	-	0.083
Afrocarribean/Asian vs Caucasian *	Empty	-	-
Age	1.00 (0.94, 1.08)	1.05	0.93
Disease duration	1.02 (0.99, 1.04)	1.63	0.278
Baseline ESR	Predicts perfectly		
Baseline CRP	0.60 (0.33, 1.08)	0.13	0.09
Baseline TJC	Predicts perfectly		
Baseline SJC	**omitted		
Baseline PtGA	0.84 (0.73, 0.98)	0.04	0.029
Baseline PhGA	Predicts perfectly		
Erosive baseline	13.50 (1.20, 152.21)	-	0.035
Female	0.67 (0.11, 3.92)	-	0.654
RF IgA positivity	0.67 (0.10, 4.36)	-	0.672
RF IgG positivity	**omitted		
RF IgM positivity	1.29 (0.07, 24.38)	-	0.867
Total PD baseline	0.64 (0.36, 1.15)	0.11	0.137
Total SH baseline	0.83 (0.65, 1.05)	0.42	0.118

5.4 Discussion

In this cohort of RA patients with low disease activity, baseline remission rates varied from 30% (Boolean remission) to 66% (DAS28 ESR remission). 47%, 33%, 22%, 23% and 10% of patients achieved sustained DAS28ESR, DAS28 CRP, SDAI, CDAI and Boolean remission over 12 months of follow up, respectively. 10% of patients were in NR. 13.5% of patients developed erosive progression. There were no differences in baseline PD and SH scores between NR and any of the remission groups. NR patients had significantly more SH at T12 compared to all 3 remission groups. There was a similar trend for PD at T12 but this did not reach significance (p value range 0.051 – 0.074).

4 other studies have reported on sustained remission. The BARFOT study group reported on sustained remission of early RA patients over 4 visits over 8 years of follow-up (Svensson et al. 2013). This study reported sustained DAS28, SDAI and Boolean remission rates of 14%, 5% and 3% respectively over 8 years. In the UK ERAS study, sustained remission as defined by DAS28 in 3 visits over 5 years was 11% (Jayakumar et al. 2012). A study of patients with established RA (BRASS cohort) patients reported only a 'minority' of patients in sustained remission regardless of the criteria used (Prince et al. 2012). The CORRONA database assessed patients with early RA with sustained CDAI remission in at least 2 consecutive visits which ranged from 60-180 days apart. The remission rate was 7%. The REMIRA study was unique since it was restricted to low disease activity states only. Our remission rates were therefore higher than these. Other remission cohorts also reported on sustained

remission rates. Cohen et al reported that 22.4% of patients achieved DAS remission (DAS <1.6) both at 3 years and 5 years and Molenaar et al reported 53% of patients were in ACR remission at baseline and at 2 years (Cohen et al. 2007, Molenaar et al. 2004). Both these studies reported remission 2 years apart. The definition of sustained remission was more stringent in the REMIRA study where patients had to be in sustained remission at all time points over 1 year.

Very few studies have explored predictors of sustained remission. The BRASS cohort reported that more males and patients with <5 years disease duration maintained remission in the first year but the survival curves were not significantly different (Prince et al. 2012). They found no differences between seropositive and seronegative patients. The ERAS study also explored predictors of sustained DAS28 remission (Jayakumar et al. 2012). Men, <6 months symptoms and TJC <10 were found to be independent predictors of sustained remission. Gender was found to be the only predictor of CDAI sustained remission (Jawaheer et al. 2012). These cohorts differed from the REMIRA cohort where predictors of sustained remission were assessed within a unique LDAS cohort. The predictors of sustained DAS28 and sustained SDAI remission in this cohort were the same and included ethnicity, ESR, CRP, TJC, patient global assessment and physician global assessment. ESR, TJC, patient global assessment and physician global assessment were also predictors of Boolean remission. In addition, erosions at baseline was also a predictor of Boolean remission. Patients who are of Afro-carribean or Asian origin are less likely to achieve sustained

remission. One potential explanation of this could be due to drug adherence and there is currently a study ongoing to investigate this (Kumar et al. 2013).

There was no difference in radiographic progression between the LDAS and sustained remission states. Even in sustained remission, radiographic progression can occur in any remission criteria. This has also been shown in another study of radiographic progression over 2 years (Lillegraven et al. 2012). The benefit of sustained remission on the prevention of radiographic progression is seen 1 year after the period of sustained remission (Aletaha et al. 2009a). There may therefore be a lag between the improvement in disease activity and radiographic outcomes, which we would not have captured during the 12 months of follow up.

The REMIRA cohort of patients had a very high proportion of patients with the presence of power Doppler signal (90%) and SH (100%). This is higher than that reported in the literature. Balsa et al reported in their cohort of remission patients 92% with SH and 42% with the presence of PD (Balsa et al. 2010). Brown et al's cohort showed 85% SH and 60% PD signal (Brown et al. 2006). It is established that the presence of a PD signal predicts radiographic progression in low disease activity states, however, the role of SH is less clear. Boyesen found SH predicted 1 year MRI erosive progression in patients with active disease (Boyesen et al. 2011b). The REMIRA study has demonstrated for the first time that SH also has a role in predicting radiographic progression in LDAS. Synovial hypertrophy was also found to be less in all sustained remission groups at the end of the study compared to the LDAS group.

This suggests that synovial hypertrophy can be reduced with stringent sustained remission.

The fact that ultrasound parameters predicted radiographic progression but not sustained remission over time may suggest that radiographic progression may not be wholly dependent on joint inflammation (Boers 2008). It has been suggested previously that the pathogenesis of RA is split into 2 distinct processes: synovial inflammation which leads to pain and swelling and synovial hypertrophy which leads to swelling and erosions (Kirwan 2004). In support of this is the finding that anti-TNF treatment has been shown to inhibit radiographic progression even in patients who are not responding clinically (Smolen et al. 2005).

The REMIRA cohort found no role for ultrasound at predicting sustained remission. This was also seen in a recent study where sustained CDAI remission was defined as remission at 2 timepoints (Gartner et al. 2013). They suggested that low-grade PD and SH signals may not necessarily reflect the presence of active synovitis in RA joints.

The treat-to target approach is the gold-standard treatment for RA. Guidelines propose intensive escalation of treatment for patients with active disease. Our study confirms that even after reaching remission, strict monitoring is required, as only a small proportion of patients achieve sustained remission. The use of clinical variables may help to stratify patients who are more likely to stay in sustained remission and help in decision making with treatment changes.

6 Serum Biomarker as predictors of sustained remission

6.1 Introduction

As discussed in the previous chapters, numerous clinical definitions of remission exist but there is still an unmet need for objective molecular biomarkers of remission that may reflect a state of true biological remission. This is especially important when drug tapering or withdrawal are being considered or when assessing the possibility of flares. Definitions of clinical remission states may be inadequate since sub-clinical synovitis may still be present. Previous studies have shown that patients in clinical remission continue to develop radiographic progression (Brown et al. 2008). Power Doppler signal has been detected in these patients and it is suggested that some patients in clinical remission continue to have subclinical disease activity. Therefore, true remission states might better be defined at a molecular level, where patients may be deemed free from subclinical disease activity.

The clinical components are subject to intra and inter assessor variability (Uhlig, Kvien & Pincus 2009, Marhadour et al. 2010) and can be confounded by co-morbidities such as fibromyalgia (Leeb et al. 2004) and joint damage. The biomarkers within these clinical disease activity measures are non-specific and can be elevated in a number of conditions eg age, anaemia, infection and malignancy. It can also be normal in patients with active disease (Keenan, Swearingen & Yazici 2008, Sokka, Pincus 2009).

A multi-biomarker disease activity algorithm using 12 different serum biomarkers has been recently validated as an objective measure of disease activity across a range of disease activity states (Curtis et al. 2012). These 12 biomarkers evaluate different aspects of pathologic pathways implicated in the pathogenesis of RA and can be broadly grouped into the following categories:

- acute-phase response (SAA, hsCRP, IL6),
- hormones (leptin and resistin),
- growth factors (VEGF and EGF), adhesion molecules (VCAM1),
- cartilage-related proteins (YKL-40) and matrix metalloproteinases (MMP1, MMP3)
- cytokine related proteins (TNFR1)

This biomarker set has been rigorously evaluated in large numbers of patients with active RA and is now available as an FDA approved assay (Vectra-DA) of disease activity in the United States. While levels of these analytes are altered in the serum of RA patients with active inflammation, it remained to be determined whether the biomarker set would have more discriminatory value at low ends of the disease spectrum than the conventional ESR or CRP level.

We also opted to include 2 further biomarkers which we felt were important in assessing disease activity in RA: CXCL10 and calprotectin.

CXCL10 has been detected in synovial fluid, synovial tissue and serum of RA patients (Hanaoka et al. 2003). Serum levels have also found to be raised in active disease and significantly reduced in response to treatment (Kuan et al. 2010). A phase 2 clinical

trial using an anti-CXCL10 monoclonal antibody (MDX-1100) has reported increased ACR20 response rate at week 12 compared to placebo group in active RA patients who have failed Methotrexate treatment (Yellin et al. 2012).

Calprotectin is expressed in RA synovial tissue (Youssef et al. 1999) and high concentrations have been found in synovial fluid and blood of RA patients (Berntzen et al. 1991). The protein has been described as a good measure of disease activity and joint inflammation in RA (Madland et al. 2002). Moreover, Calprotectin was found to be an independent predictor of radiograph damage cross-sectionally and longitudinally (Hammer et al. 2007, Hammer et al. 2010).

The role of calprotectin in the remission state has been explored in juvenile idiopathic arthritis (JIA) patients. It has been shown to be an important predictor for relapse in patients after treatment withdrawal (Foell et al. 2010, Gerss et al. 2012). In early RA, normalisation of calprotectin levels have been reported in patients with treatment. It was found to be a predictive marker for improvement in swollen joints (Andres Cerezo et al. 2011).

In this present study, we studied a cohort of clinically similar group of patients with low disease activity states (LDAS) including remission. We aimed firstly to define a molecular signature of point remission and sustained remission and secondly to explore the role of these biomarkers in predicting sustained remission. This work was

undertaken as part of a collaboration with investigators at Crescendo Biosciences, following a short secondment to their unit in San Francisco.

6.2 Methods and Patients

6.2.1 REMIRA cohort and clinical assessments

This has been discussed in Chapter 5.

6.2.2 Serum biomarkers

Sera were collected at all study visits using standard serum separator tubes according to the manufacturer's instructions. The sera were frozen at -80 Celsius within 6 hours of blood sampling. Due to limited resources, 3 timepoints were used to measure the serum biomarkers. Baseline, 3 months and 6 months were chosen as predictors of sustained remission at 12 months. 3 patients declined blood sampling therefore 101 patients have available serum. All 14 biomarkers were measured by Crescendo Bioscience laboratories.

The development of the MBDA score was discussed in the introduction (1.7.8). The concentrations of 12 serum proteins—serum amyloid A (SAA), IL-6, TNF receptor superfamily member 1A (TNF-R1), VEGFA, MMP1, human cartilage glycoprotein 39 (YKL40), MMP3, epithelial growth factor (EGF), vascular cell adhesion molecule 1 (VCAM1), leptin, resistin and CRP—were measured by customized immunoassays, quantified on a Sector Imager 6000 (Meso Scale Discovery, Gaithersburg, MD, USA) and transformed to the power 0.1 to achieve approximately normal distributions.

CXCL10 and Calprotectin were measured using commercially available ELISA kits according to the manufacturers' protocols. The Calprotectin assay was from Buhlmann (MRP 8/14 ELISA Product Code EK-MRP8/14). For CXCL10, a modified version of the R&D Systems Human CXCL10/IP-10 Quantikine ELISA (Product Code: DIP100) was used. For this ELISA assay, in-house manufactured pre-diluted standards and controls were used. A 2-fold dilution of samples was carried out. The rest of the procedure was performed per the manufacturers protocol

6.2.3 RA reference cohort for the MBDA score

The RA reference cohort was called InFoRM (Index for Rheumatoid Arthritis Measurement), an observational study across multiple centers in North America (collected by Crescendo). Any patients of age 18-90 that were diagnosed of RA by ACR1987 criteria and able to consent were eligible to participate. There were approximately 1000 patients enrolled. All of them received routine care. The reference cohort consisted of 512 patients that had clinical characteristics representative of the entire study population. They were used in one of the steps for training Vectra DA algorithm. The average age was 59, and 76% were female. The mean SJC28 and TJC28 were 4.3 and 5.5, respectively.

6.2.4 Statistical analysis

STAT 11.2 (StataCorp, College Station, TX, USA) was used for statistical analysis. Remission at baseline was defined as DAS28ESR <2.6, SDAI ≤3.3, or MBDA score ≤ 25

(range 0-100) at baseline. Low disease activity states (LDAS) is defined as no remission by any clinical criteria. Sustained remission (SR) is defined as achieving remission at all time-points, intermittent remission (IR) is defined as achieving remission in at least 1 time-point but not all. No remission (NR) is defined as not achieving remission at any time-point by any criteria. Individual variables were assessed descriptively as median values and interquartile ranges (IQR). The difference between remission and non-remission was assessed using Mann-Whitney test and between NR/IR/SR remission using Kruskal-Wallis test. Pairwise comparisons were also made using Mann-Whitney U test. All reported p values are 2-sided.

Agreement between the different remission criteria was assessed using Cohen's kappa test. The area under the curve from the receiver-operating characteristic (ROC) was used to assess the different remission criteria ability to discriminate between patients in Boolean remission and non-remission at baseline or none and sustained remission over the 1 year follow-up.

Time-integrated values were calculated using area under the curve (AUC). These were computed using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA) using the trapezoidal method. The last observation carried forward method was used to handle missing data. AUC values were calculated for all the 14 biomarkers and the MBDA score for the first 3 visits (6 months).

Univariate logistic regression models were used to estimate the associations between variables as potential predictors of sustained remission. The odds ratio and its 95%

confidence interval (95% CI) represent the increased or decreased risk associated with a 1-unit change in the predictor variable for continuous variables. For dichotomous variables, the odds ratio represents the risk associated with having the characteristic compared with not having it. In continuous variables, it can be difficult to interpret the actual differences amongst variables because variables can be scaled in different units; for example, a 1-unit change in 1 biomarker in $\mu\text{g/ml}$ is not comparable with another 1-unit change in ng/ml . The standardized odds ratio, or the odds ratio per 1-SD change, allows comparison among predictor variables using common units. Standardised OR is defined as $\text{Exp}(B \times \text{SD})$ where B = B co-efficient and SD = standard deviation.

6.3 Results

6.3.1 Patient Cohort

The patient characteristics of the REMIRA cohort has been described in the previous chapter.

6.3.2 Biomarker summary

Serum samples were available in 101 REMIRA patients. Table 6.1 summarised the median values (IQR) of all 14 biomarkers at baseline and area under the curve values (AUC). Figure 6.1 shows the distribution of the normalised biomarker levels of the 12 biomarkers from the MBDA panel at baseline. This was calculated using the median values according to a reference RA cohort whose disease spans the whole spectrum of

disease activity. Within the REMIRA cohort, SAA levels were the lowest and EGF was the highest when compared to the reference cohort. EGF is reported as being inversely correlated to disease activity (through personal communication with Crescendo Bioscience). With the exception of EGF, the median values of the REMIRA cohort were below 1 showing that it is lower end of the RA range. Figure 6.2 is a heatmap showing the percentile of the biomarkers at baseline. This demonstrates that on a molecular level, there is marked heterogeneity within this cohort of patients inspite of their relatively homogenous clinical phenotype.

Table 6-1 : Summary of MBDA and Individual Biomaker Levels
Within the REMIRA cohort (n=101). Median values (IQR) of Biomarkers at baseline and AUC over the first 6 months

Biomarker	Median Baseline values (IQR)	Median AUC(IQR)
MBDA (0-100)	29 (18- 40)	180 (126 – 229)
EGF (pg/ml)	254.44 (181.71-367.09)	1687 (1148 – 2153)
IL6 (pg/ml)	9.25 (5.86 – 16.30)	63 (40-96)
Leptin (ng/ml)	9.33 (3.81 – 27.69)	64 (26 – 172)
MMP1 (ng/ml)	6.39 (4.55 – 9.30)	41 (28 – 56)
MMP3 (ng/ml)	26.11 (17.25 – 39.16)	157 (111 – 215)
Resistin (ng/ml)	7.37 (5.97 – 8.79)	46 (39 – 59)
SAA (µg/ml)	1.70 (0.94 – 3.45)	11 (5 – 23)
hsCRP (mg/L)	2.36 (1.10 – 6.27)	17 (7 – 36)
TNFR1 (ng/ml)	1.51 (1.32 – 2.02)	10 (8-12)
VCAM1 (ng/ml)	559.91 (462.50 -671.61)	3065 (2607 – 3664)
VEGF (pg/ml)	267 (176 – 408)	1548 (1104-2640)
YKL-40 (ng/ml)	61.55 (41.33 – 97.96)	364 (238 – 698)
Calprotectin (ng/ml)	2289.55 (1507.45 – 3792.78)	14106 (11324 – 18251)
CXCL10 (pg/ml)	204.89 (143.84 - 331.36)	1293 (918 - 1968)

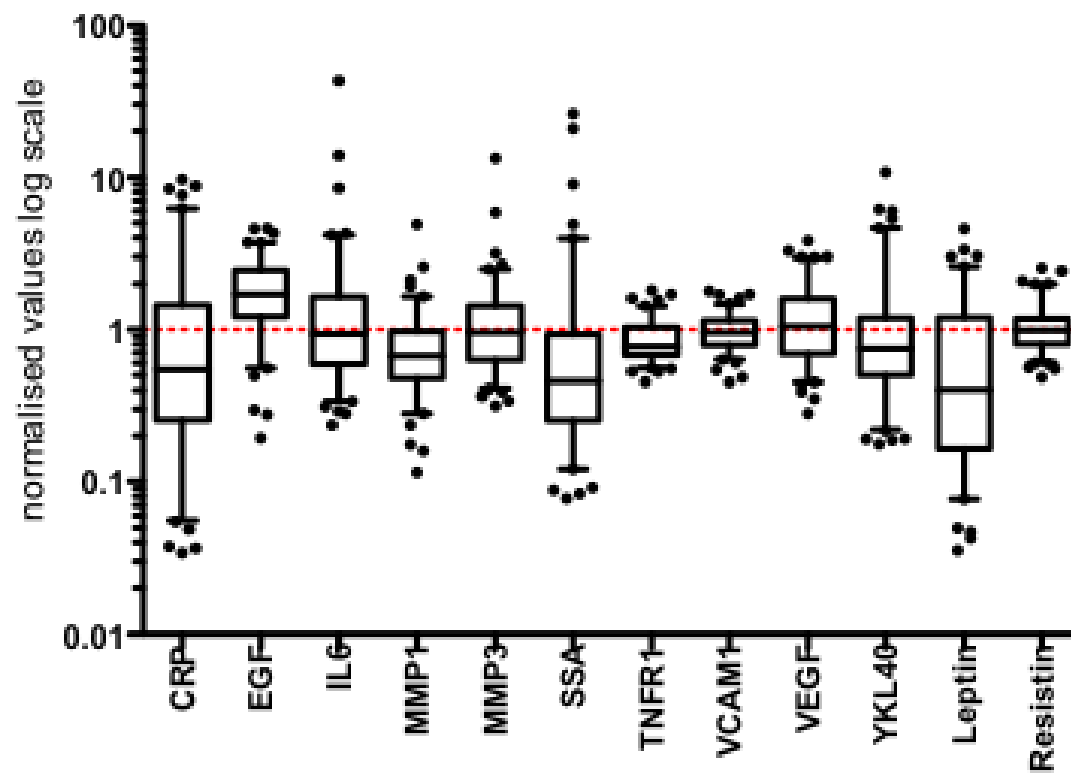


Figure 6-1: Normalised Median Biomarker Levels

Median (IQR) Biomarker levels (error bars 5-95 centiles). Normalised by median values of reference cohort.

Dotted line across 1 is the median value of the reference cohort

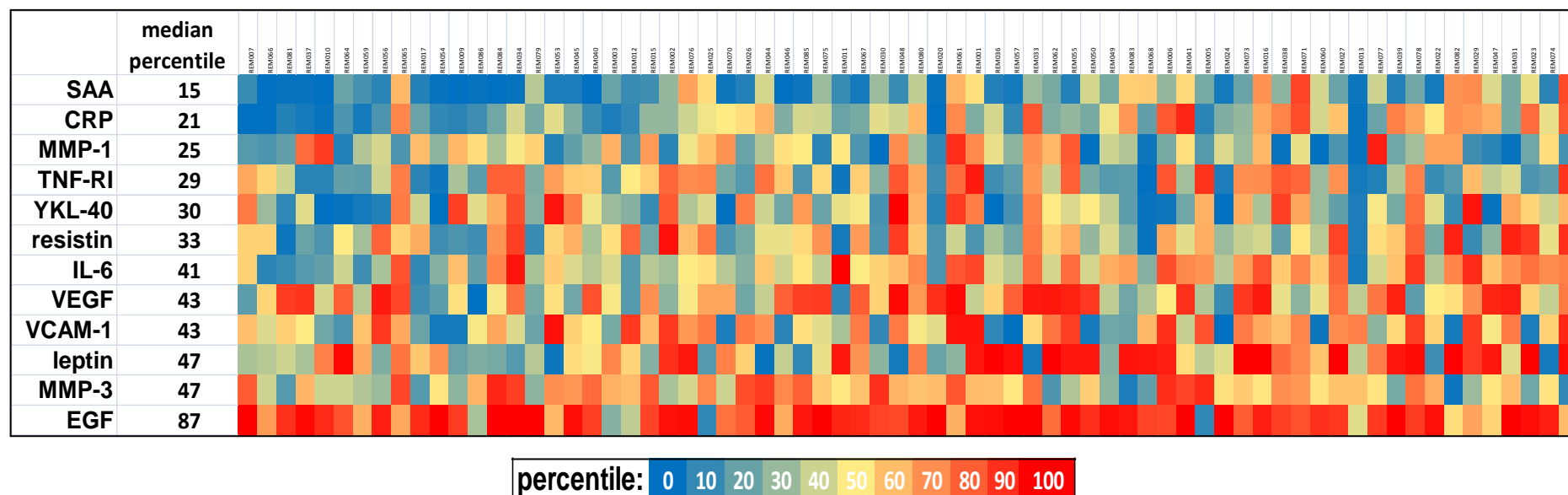


Figure 6-2: The REMIRA Cohort Biomarker Levels as Percentile Levels (n = 70).

Each column represents a patient, each row a biomarker. Patients are arranged in order of increasing disease activity and biomarkers are arranged in order of increasing median concentrations relative to reference cohort. (median percentile shown in brackets). Figure generated in collaboration with Crescendo Bioscience.

6.3.3 Agreement between MBDA remission and clinical remission criteria

Agreement between the clinical remission criteria have been discussed in the previous chapter. Table 6.2 shows the agreement between MBDA remission and the clinical remission criteria at baseline ranging from 61% to 66%. The weakest agreement is with DAS28CRP and best is with DAS28ESR. The agreement of sustained MBDA remission over 6 months with the different sustained remission criteria (over 1 year) is better than point remission ranging from 75% to 81% (Table 6.3). The worst agreement is with DAS28CRP and the best is with Boolean.

Table 6-2: Agreement Between the Clinical Remission Criteria and MBDA
Using Cohen's kappa test.

	Boolean	SDAI	CDAI	DAS28CRP	DAS28ESR
SDAI	85%				
CDAI	84%	99%			
DAS28CRP	70%	86%	87%		
DAS28ESR	60%	69%	69%	80%	
MBDA	66%	64%	63%	61%	65%

Table 6-3 Agreement Between Sustained Remission and MBDA
Using Cohen's kappa test. *MBDA score – sustained remission over 3 timepoints in the first 6 months

	Boolean	SDAI	CDAI	DAS28CRP	DAS28ESR
SDAI	88%				
CDAI	87%	99%			
DAS28CRP	77%	89%	90%		
DAS28ESR	63%	71%	72%	80%	
MBDA*	81%	77%	77%	75%	79%

6.3.4 Performance of MBDA remission

The performance of the MBDA score at differentiating between remission and non-remission states using the different clinical remission criteria are shown in table 6.4. AUROC ranged from 0.62 for CDAI to 0.71 for DAS28 ESR. Table 6.5 shows the AUROC values for sustained MBDA remission over 6 months at differentiating the different non-sustained clinical remission and sustained remission over 1 year. The values ranged from 0.58 to 0.71. The MBDA remission was the least effective at differentiating between DAS28CRP remission and non-remission and better at differentiating between Boolean remission and non-remission. Since CDAI was very similar to SDAI, this was excluded from further analysis. In addition, DAS28CRP agreement and performance were the least discriminatory; therefore this was also excluded from further analysis.

Table 6-4: Ability of MBDA to Differentiate Between baseline Remission and NR
NR= non-remission

Remission Criteria	AUROC	95% CI
DAS28ESR	0.71	0.63 – 0.79
DAS28CRP	0.63	0.54 – 0.73
SDAI	0.63	0.54 – 0.73
CDAI	0.62	0.73 – 0.72
Boolean	0.66	0.55 – 0.76

Table 6-5: Ability of MBDA over 6 months to Differentiate Between SR and NR
SR= sustained remission, NR= non-remission

Remission Criteria	AUROC	95% CI
DAS28ESR	0.61	0.55 – 0.68
DAS28CRP	0.58	0.50 – 0.66
SDAI	0.64	0.54 – 0.75
CDAI	0.63	0.53– 0.73
Boolean	0.71	0.54– 0.87

6.3.5 Baseline Biomarker levels and point remission

Table 6.6 shows the median (IQR) values of all 14 biomarkers in the LDAS group (not in remission by any clinical remission criteria) and remission groups (DAS28ESR, SDAI and Boolean remission). IL6, Leptin, SAA, hsCRP and total MBDA score were all significantly lower in all 3 remission groups when compared to LDAS group. In addition, TNFR1 and VCAM1 were also significantly lower in DAS28ESR group when compared to LDAS.

6.3.6 Baseline biomarker levels and sustained remission.

The median (IQR) baseline biomarker levels between No remission (NR), intermittent remission (IR) and sustained remission (SR) are shown in Table 6.7. DAS28, SDAI and Boolean remission groups are shown. CXCL10, IL6, Leptin, SAA, hsCRP and MBDA scores were significantly different in all remission criteria groups when compared to LDAS. The exception of this is leptin within the SDAI groups. Calprotectin was not significantly different in any of the remission groups. Figures 6.3 -6.5 and 6.9 show the pairwise comparisons between NR vs IR, NR vs SR and IR vs SR for these individual biomarkers and the MBDA score respectively. In DAS28 groups, there was no significant difference between NR and DAS28 IR groups (Figure 6.3). This may be due to the large error bars in the DAS28 IR group suggesting that there is more heterogeneity within this group. In SDAI and Boolean groups, there were no significant differences between IR and SR groups. With leptin, there was a trend towards a decrease between NR and IR but the p value just greater than 0.05 (Fig 6.4 and 6.5).

Table 6-6: Baseline Biomarker Values in LDAS and Remission at Baseline
Comparing low disease activity (LDAS) to DAS28ESR, SDAI and Boolean remission groups at baseline.
Values in median (IQR), Levels of significance determined by Mann Whitney test.

Biomarkers	LDAS	DAS28ESR	p value	SDAI	p value	Boolean	p value
<i>EGF (pg/ml)</i>	270 (185, 342)	264 (197, 376)	0.653	230 (162, 364)	0.685	256 (184, 373)	0.832
<i>IL6 (pg/ml)</i>	13 (10, 24)	7 (5, 13)	<0.0001	6.61 (4.79, 9.96)	<0.0001	6.05 (4.36, 8.72)	<0.0001
<i>Leptin (ng/ml)</i>	16 (5, 35)	7 (4, 16)	0.036	4.96 (3.35,13.52)	0.008	4.96 (3.27, 10.64)	0.01
<i>MMP1 (ng/ml)</i>	6.01 (4.36, 8.99)	6.56 (5.07, 948)	0.617	7.38 (5.13, 10.05)	0.361	7.07 (5.19, 1.06)	0.404
<i>MMP3 (ng/ml)</i>	26 (18, 36)	27 (20, 40)	0.804	27 (17, 39)	0.982	27 (17, 41)	0.988
<i>Resistin (ng/ml)</i>	7.43 (5.97, 11.80)	7.08 (5.91, 8.18)	0.384	7.19 (5.91, 8.70)	0.685	7.52 (5.97, 8.75)	0.693
<i>SAA (μl/ml)</i>	3.19 (1.71, 5.98)	1.20 (6.73, 2.21)	<0.0001	1.11 (6.27, 2.04)	<0.0001	1.11 (0.64, 1.79)	<0.0001
<i>TNFR1 (ng/ml)</i>	1.76 (1.40, 2.29)	1.45 (1.30, 1.90)	0.027	1.50 (1.33, 1.99)	0.105	1.53 (1.34, 1.98)	0.154
<i>VCAM1 (ng/ml)</i>	602 (539, 729)	531 (449, 627)	0.018	546 (445, 637)	0.053	558 (462, 646)	0.118
<i>VEGF (pg/ml)</i>	291 (198, 453)	226 (165, 393)	0.209	247 (177, 403)	0.338	271 (179, 407)	0.575
<i>YKL40 (ng/ml)</i>	83 (43, 96)	53 (35, 96)	0.121	60 (37, 14)	0.535	60 (35, 105)	0.332
<i>hsCRP (mg/ml)</i>	5.10 (1.69, 13.20)	1.46 (0.63, 2.87)	<0.0001	1.56 (0.68, 2.61)	<0.0001	1.46 (0.46, 2.51)	<0.0001
<i>MBDA score (0-100)</i>	37 (25, 50)	22 (16, 33)	<0.0001	21 (16, 32)	<0.0001	18 (15, 31)	<0.0001
<i>Calprotectin (ng/ml)</i>	2998 (1978, 5130)	1998 (1450, 3331)	0.011	1937 (1451, 3160)	0.011	1848 (1450, 3190)	0.017
<i>CXCL10 (pg/ml)</i>	256 (195, 416)	175 (127, 236)	<0.0001	183 (133, 245)	0.001	184 (132, 231)	0.001

Table 6-7 Baseline biomarker values in NR and remission over 12 months
Comparing no remission by any criteria (NR) to DAS28ESR, SDAI and Boolean intermittent (IR) and sustained remission (SR) groups.
Values in median (IQR), Levels of significance determined by Kruskal-Wallis test.

	NR	DAS28-IR	DAS28-SR	P value	SDAI-IR	SDAI-SR	P value	Boolean-IR	Boolean-SR	P value
EGF (pg/ml)	358 (227, 417)	233 (180, 334)	256 (188, 371)	0.376	246 (184, 356)	249 (145, 362)	0.374	239 (184, 356)	264 (145, 397)	0.398
IL6 (pg/ml)	14 (11, 31)	12 (6, 21)	7 (4, 11)	0.001	8.45 (5.97, 13.61)	6.12 (4.20, 10.21)	0.008	6.65 (4.80, 11.09)	6.79 (4.24, 9.33)	0.009
Leptin (ng/ml)	23 (8, 62)	14 (5, 34)	6 (3, 15)	0.003	7.39 (3.70, 24)	4.65 (3.27, 13.94)	0.081	6.97 (3.55, 2.26)	4.45 (2.97, 8.83)	0.045
MMP1 (ng/ml)	5.24 (3.68, 8.90)	6.56 (4.87, 9.32)	6.18 (4.47, 9.16)	0.613	7.43 (5.46,10.47)	6.32 (4.49, 8.51)	0.421	6.31 (5.07, 10.47)	7.15 (4.60, 8.51)	0.745
MMP3 (ng/ml)	30 (17, 42)	23 (17, 34)	27 (19, 41)	0.524	27 (20, 41)	26 (16, 33)	0.491	27 (17, 40)	25 (15, 45)	0.935
Resistin (ng/ml)	11 (6, 14)	7 (6, 9)	7 (6, 8)	0.274	6.98 (5.67, 7.92)	7.99 (5.84, 9.88)	0.073	7.02 (5.84, 8.80)	8.149 (7.58, 9.88)	0.148
SAA (ug/ml)	3.89 (2.24, 16.50)	1.88 (1.15, 4.37)	1.18 (0.66, 2.32)	0.001	1.76 (0.67, 3.15)	1.14 (0.63, 2.06)	0.013	1.434 (0.70, 2.13)	1.08 (6.28, 2.21)	0.016
TNFR1 (ng/ml)	1.66 (1.39, 2.03)	1.58 (1.33, 2.08)	1.47 (1.31, 1.94)	0.638	1.41 (1.20, 1.79)	1.50 (1.35, 2.03)	0.368	1.48 (1.34, 2.14)	1.73 (1.37, 2.03)	0.884
VCAM1 (ng/ml)	577 (469, 651)	583 (418, 710)	546 (463, 626)	0.697	541 (465, 645)	557 (458, 671)	0.896	557 (418, 646)	545 (462, 685)	0.835
VEGF (pg/ml)	403 (247, 519)	245 (177, 384)	261 (171, 408)	0.299	232 (177, 398)	275 (150, 408)	0.276	210 (159, 393)	208 (137, 310)	0.112
YKL40 (ng/ml)	95 (53, 124)	58 (41, 91)	59 (40, 102)	0.59	53 (38, 70)	61 (32, 134)	0.351	62 (35, 98)	60 (48, 135)	0.642
hsCRP (mg/ml)	5.91 (3.31, 11.70)	3.01 (1.45, 7.76)	1.46 (0.71, 2.76)	0.002	1.69 (1.04, 3.57)	1.46 (0.46, 2.55)	0.014	2.27 (0.78, 3.23)	1.30 (0.21, 2.55)	0.016
MBDA score	41 (32, 52)	34 (20, 45)	23 (16, 33)	<0.0001	28 (18, 35)	20 (15, 37)	0.012	25 (17, 37)	17 (15, 40)	0.015
Calprotectin (ng/ml)	3278 (2020, 5470)	2377 (1641, 3825)	2007 (1452, 3332)	0.23	2007 (1531,3132)	1962 (1348,3190)	0.203	2055 (1504,3793)	2945 (1783,3190)	0.347
CXCL10 (pg/ml)	297 (229, 379)	232 (162, 395)	183 (124, 234)	0.002	175 (131, 28)	220 (133, 258)	0.046	184 (139, 240)	228 (103, 258)	0.025

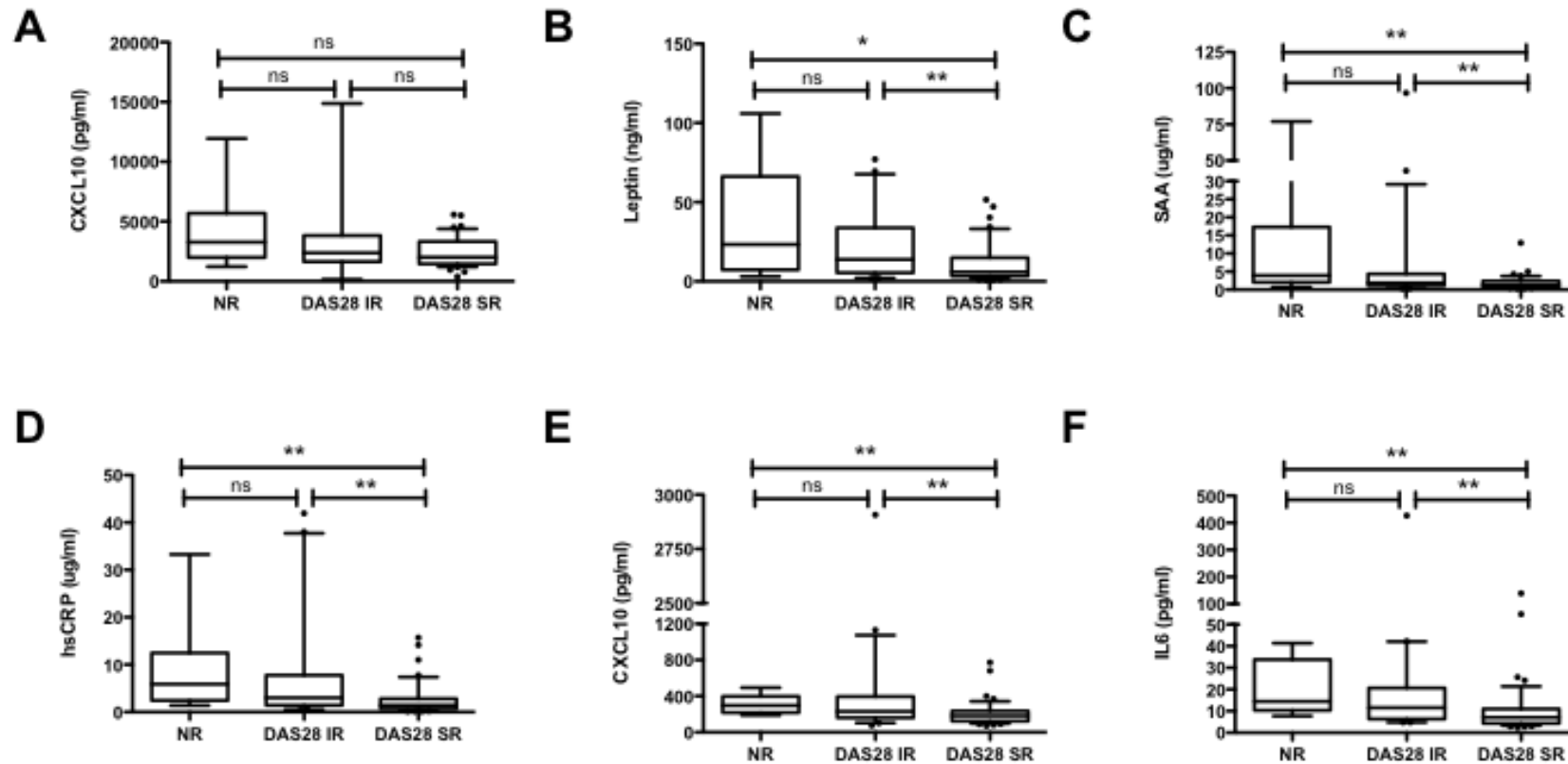


Figure 6-3 Baseline Biomarkers Values in NR, DAS28 IR and SR Groups

A = Calprotectin (ng/ml), B = Leptin (ng/ml), C = SAA (μg/ml), D = hsCRP (μg/ml), E = CXCL10 (pg/ml), F = IL6 (pg/ml). Values expressed as medians with IQR. NR = no remission by any criteria, IR = intermittent remission, SR = sustained remission. Levels of significance determined by Mann-Whitney test (NR vs IR, IR vs SR, NR vs SR). ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$

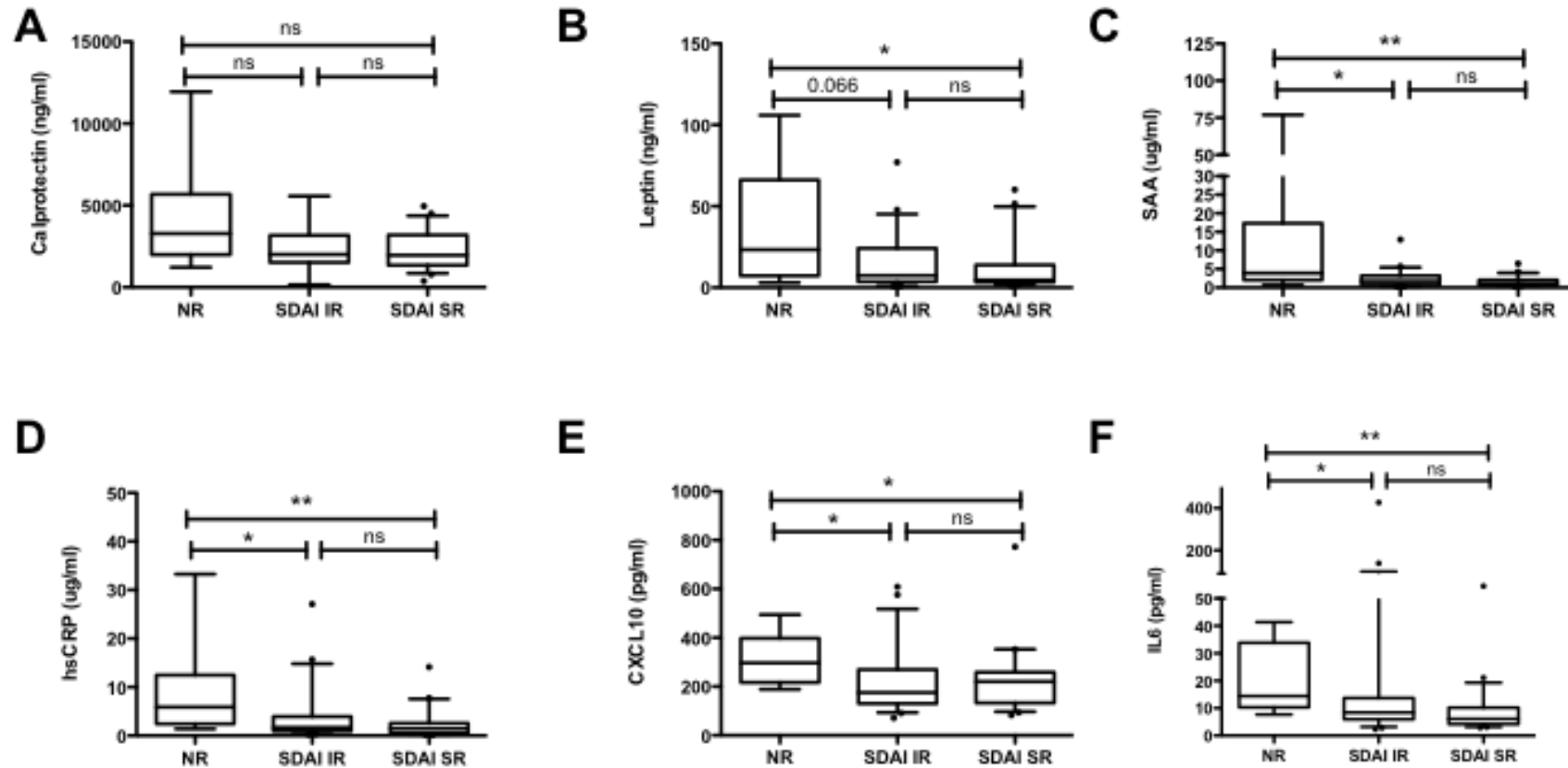


Figure 6-4: Baseline Biomarkers Values in NR, SDAI IR and SR Groups

A = Calprotectin (ng/ml), B = Leptin (ng/ml), C = SAA ($\mu\text{g/ml}$), D = hsCRP ($\mu\text{g/ml}$), E = CXCL10 (pg/ml), F = IL6 (pg/ml). Values expressed as medians with IQR. NR = no remission by any criteria, IR = intermittent remission, SR = sustained remission. Levels of significance determined by Mann-Whitney test (NR vs IR, IR vs SR, NR vs SR). ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$

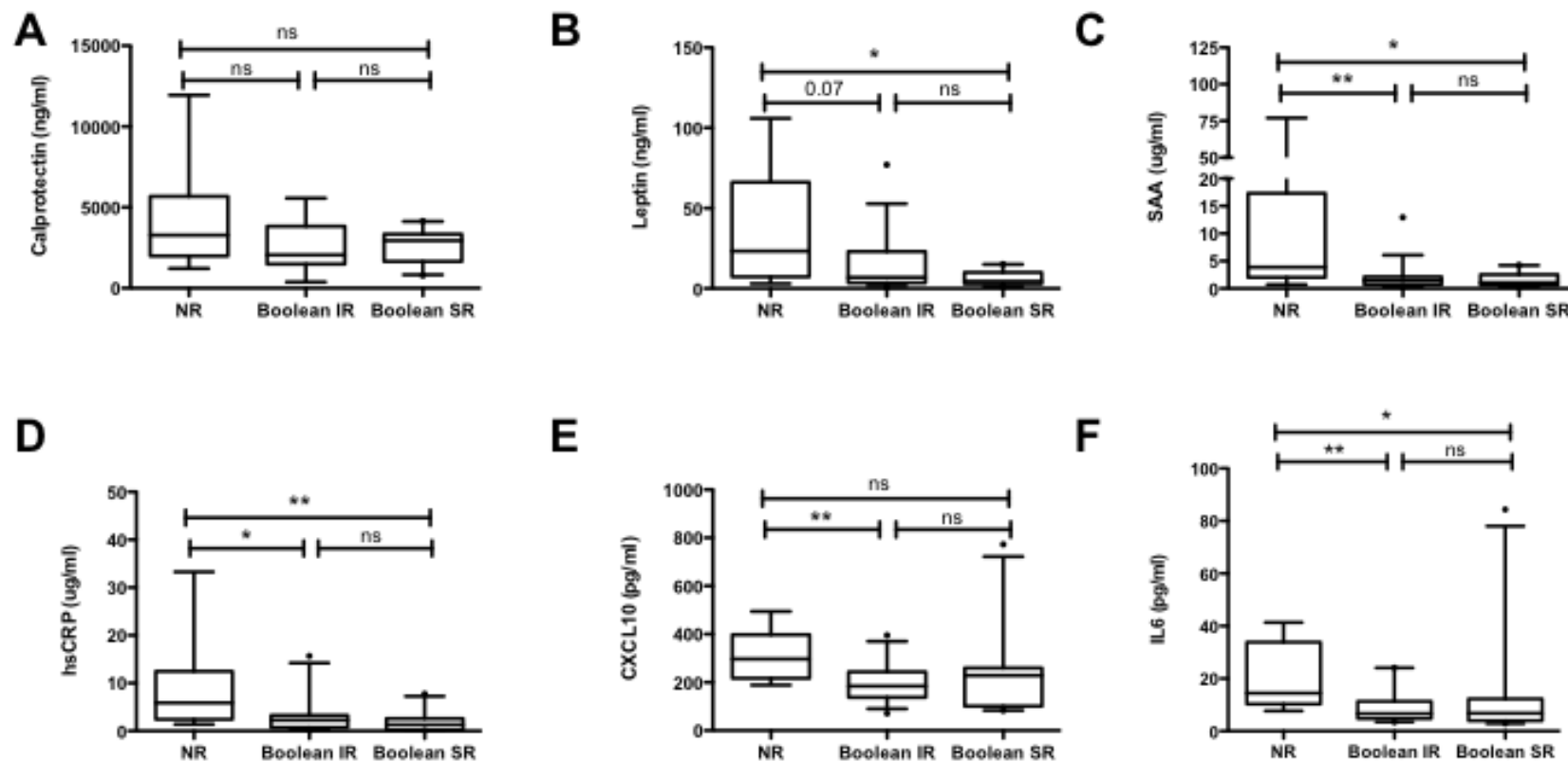


Figure 6-5: Baseline Biomarkers Values in NR, Boolean IR and SR Groups.

A = Calprotectin (ng/ml), B = Leptin (ng/ml), C = SAA ($\mu\text{g/ml}$), D = hsCRP ($\mu\text{g/ml}$), E = CXCL10 (pg/ml), F = IL6 (pg/ml). Values expressed as medians with IQR. NR = no remission by any criteria, IR = intermittent remission, SR = sustained remission. Levels of significance determined by Mann-Whitney test (NR vs IR, IR vs SR, NR vs SR). ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$

6.3.7 AUC biomarker levels and sustained remission

The values of the biomarkers over 6 months were combined into a time-integrated value using AUC. The median (IQR) AUC values between NR, IR and SR are shown in Table 6.8 and Figures 6.6 - 6.9. AUC values for Calprotectin CXCL10, IL6, Leptin, SAA and hsCRP and MBDA scores were all significantly different between these groups using all 3 remission criteria (Table 6.8). Again, the exception was with leptin AUC levels in the SDAI groups and calprotectin in the Boolean groups where the p values were just over 0.05. Pairwise comparisons showed similar trends as with baseline biomarker values (Figures 6.6 - 6.9).

Table 6-8 AUC Biomarkers Values Between NR, IR and SR groups

*Values expressed as medians with IQR. NR= no-remission by any criteria, IR = intermittent remission, SR = sustained remission.
Kruskall-Wallis test preformed.*

	NR	DAS28-IR	DAS28-SR	P value	SDAI-IR	SDAI-SR	P value	Boolean-IR	Boolean-SR	value
EGF AUC	1908 (1387, 2314)	1529 (1144,2124)	1680 (1035, 2142.5)	0.656	1715 (1144, 2162)	1675 (687, 1946)	0.59	1466 (1110, 1847)	1743 (652, 1946)	0.41
IL6 AUC	91.56 (61.28, 191.35)	79.96 (43.2,109.6)	47.42 (32.69, 65.01)	0.001	60.29 (37.87, 85.14)	41.58 (26.23, 63.48)	0.021	46.52 (36.87, 72.7)	40.82 (21.97, 53.48)	0.019
Leptin AUC	123.69 (54.45, 390.05)	91.84 (32.16, 233.3)	38.35 (20.12, 85.18)	0.007	43.81 (27.98, 139.5)	33.31 (17.03, 71.91)	0.068	38.75 (24.03, 151.3)	27.96 (17.03, 57.57)	0.044
MMP1 AUC	37.855 (23.49, 48.2)	43.39 (31.83, 54.88)	38.91 (26.32, 56.7)	0.625	45.12 (31.83, 58.35)	37.01 (26.31, 50.93)	0.414	37.92 (29.57, 56.94)	41.43 (30.23, 50.51)	0.865
MMP3 AUC	167.25 (105.47, 233)	143.5 (99.38, 209.4)	163.15 (123.5, 216.35)	0.73	164 (113.1, 236.4)	146.5 (95.9, 202.6)	0.536	151.3 (119.3, 213.4)	168.65 (95.57, 215.4)	0.998
Resistin AUC	72.25 (43.63, 81.95)	46.29 (37.56, 59.53)	43.92 (39.05, 54.025)	0.089	44.375 (36.94, 50.6)	47.4 (39.5, 59.29)	0.091	43.92 (36.94, 54.12)	49.68 (40.11, 59.29)	0.137
SAA AUC	27.43 (19.69, 76.79)	12.48 (5.70, 31.18)	7.56 (4.46,14.95)	0.001	10.24 (5.61, 16.12)	5.61 (4.13, 17.19)	0.01	9.59 (5.42, 15.87)	4.68 (3.54, 14.2)	0.007
TNFR1 AUC	10.53 (8.20, 11.59)	9.51 (8.14, 12.55)	9.28 (8.25, 12.05)	0.845	9.134 (7.98, 12.34)	9.54 (8.39, 12.53)	0.734	9.31 (8.2, 12.34)	10.12 (8.53, 12.53)	0.908
VCAM1 AUC	3260 (2614, 3595)	3246 (2619, 4060)	2886 (2548, 3509)	0.338	3004 (2603, 3537)	2874 (2492, 3656)	0.922	2871 (2619, 3617)	2886 (2345, 3877)	0.932
VEGF AUC	2215 (1424, 3024)	1395 (1087, 2595)	1548 (1099, 2670)	0.362	1351 (1064, 2767)	1582 (824, 2567)	0.324	1260 (1015, 2304)	1282 (802, 1846)	0.139
YKL40 AUC	499 (276, 893)	448 (239, 679)	333 (231, 679)	0.697	332 (230, 596)	339 (206, 793)	0.698	351 (209, 657)	332 (260, 725)	0.763
hsCRP AUC	36.84 (22.66, 59.09)	20.19 (9.68, 57.65)	12.36 (4.59, 24.52)	0.002	12.69 (6.56, 27.33)	8.28 (3.25, 26.18)	0.014	13.84 (6.428, 28.8)	5.57 (2.82, 17.03)	0.006
MBDA score AUC	240 (215, 308)	194 (140, 261)	152 (101, 200)	0.001	171 (128, 198)	132 (92, 222)	0.008	157 (126, 198)	96 (90, 207)	0.009
Calprotectin AUC	27651 (13779, 33266)	15903 (11401, 20498)	12425 (9837, 16380)	0.007	13157 (11324, 17578)	12102 (8248, 14505)	0.02	12517 (10754, 16451)	13465 (11539, 16491)	0.054
CXCL10 AUC	1780 (1566, 2172)	1354 (943, 2886)	1153 (724, 1419)	0.003	1127 (758, 1649)	1346 (620, 1961)	0.046	1150 (750, 1411)	1389 (592,1484)	0.031

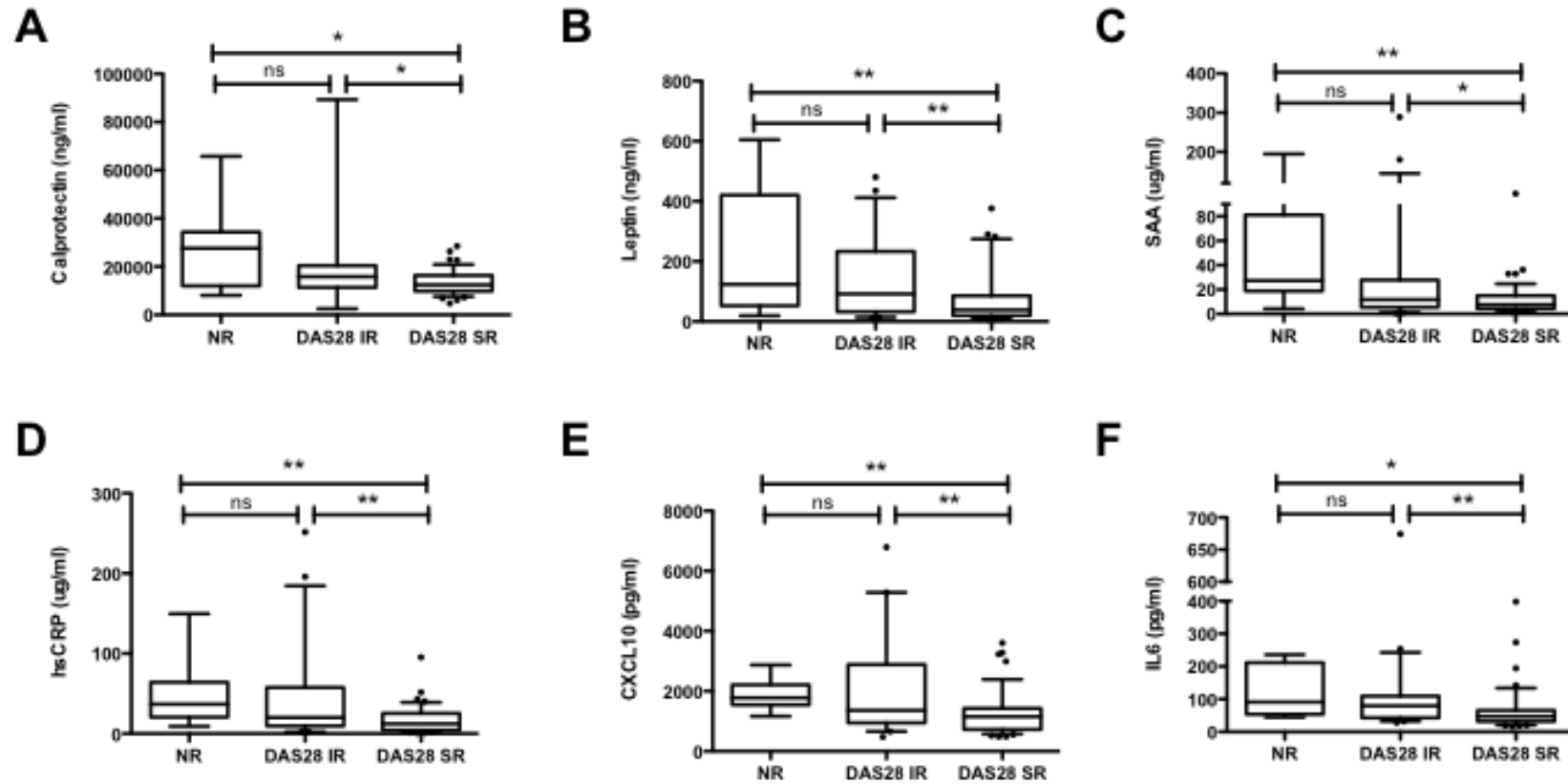


Figure 6-6 AUC Biomarker Values in NR, DAS28 IR and SR Groups

A = Calprotectin (ng/ml), B = Leptin (ng/ml), C = SAA (μg/ml), D = hsCRP (μg/ml), E = CXCL10 (pg/ml), F = IL6 (pg/ml). Values expressed as medians with IQR. NR = no remission by any criteria, IR = intermittent remission, SR = sustained remission. Levels of significance determined by Mann-Whitney test (NR vs IR, IR vs SR, NR vs SR). ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$

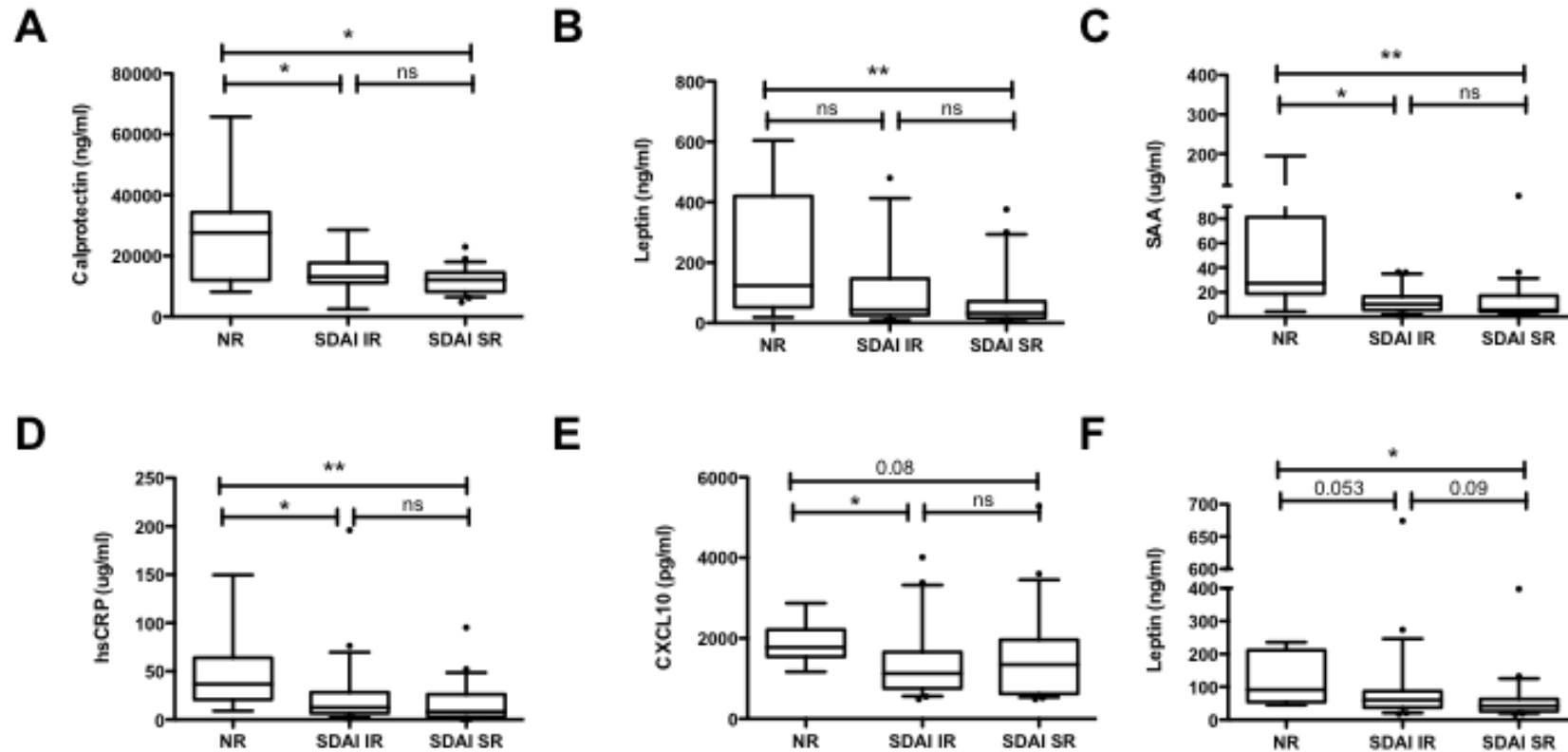


Figure 6-7 AUC Biomarker Values in NR, SDAI IR, SR Groups

A = Calprotectin (ng/ml), B = Leptin (ng/ml), C = SAA ($\mu\text{g/ml}$), D = hsCRP ($\mu\text{g/ml}$), E = CXCL10 (pg/ml), F = IL6 (pg/ml). Values expressed as medians with IQR. NR = no remission by any criteria, IR = intermittent remission, SR = sustained remission. Levels of significance determined by Mann-Whitney test (NR vs IR, IR vs SR, NR vs SR). ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$

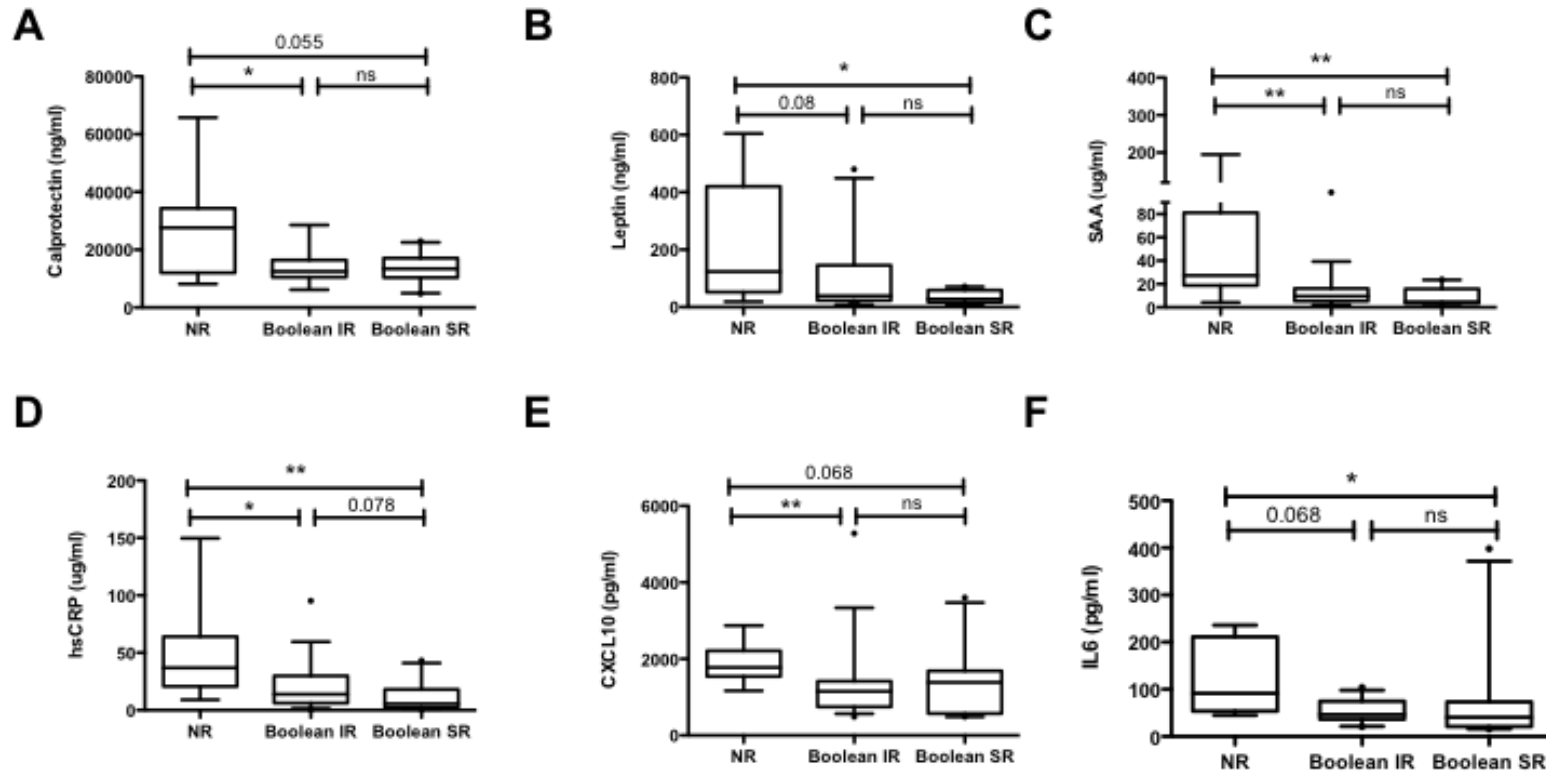


Figure 6-8 AUC Biomarker Values in NR, Boolean IR and SR Groups.

A = Calprotectin (ng/ml), B = Leptin (ng/ml), C = SAA ($\mu\text{g/ml}$), D = hsCRP ($\mu\text{g/ml}$), E = CXCL10 (pg/ml), F = IL6 (pg/ml). Values expressed as medians with IQR. NR = no remission by any criteria, IR = intermittent remission, SR = sustained remission. Levels of significance determined by Mann-Whitney test (NR vs IR, IR vs SR, NR vs SR). ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$

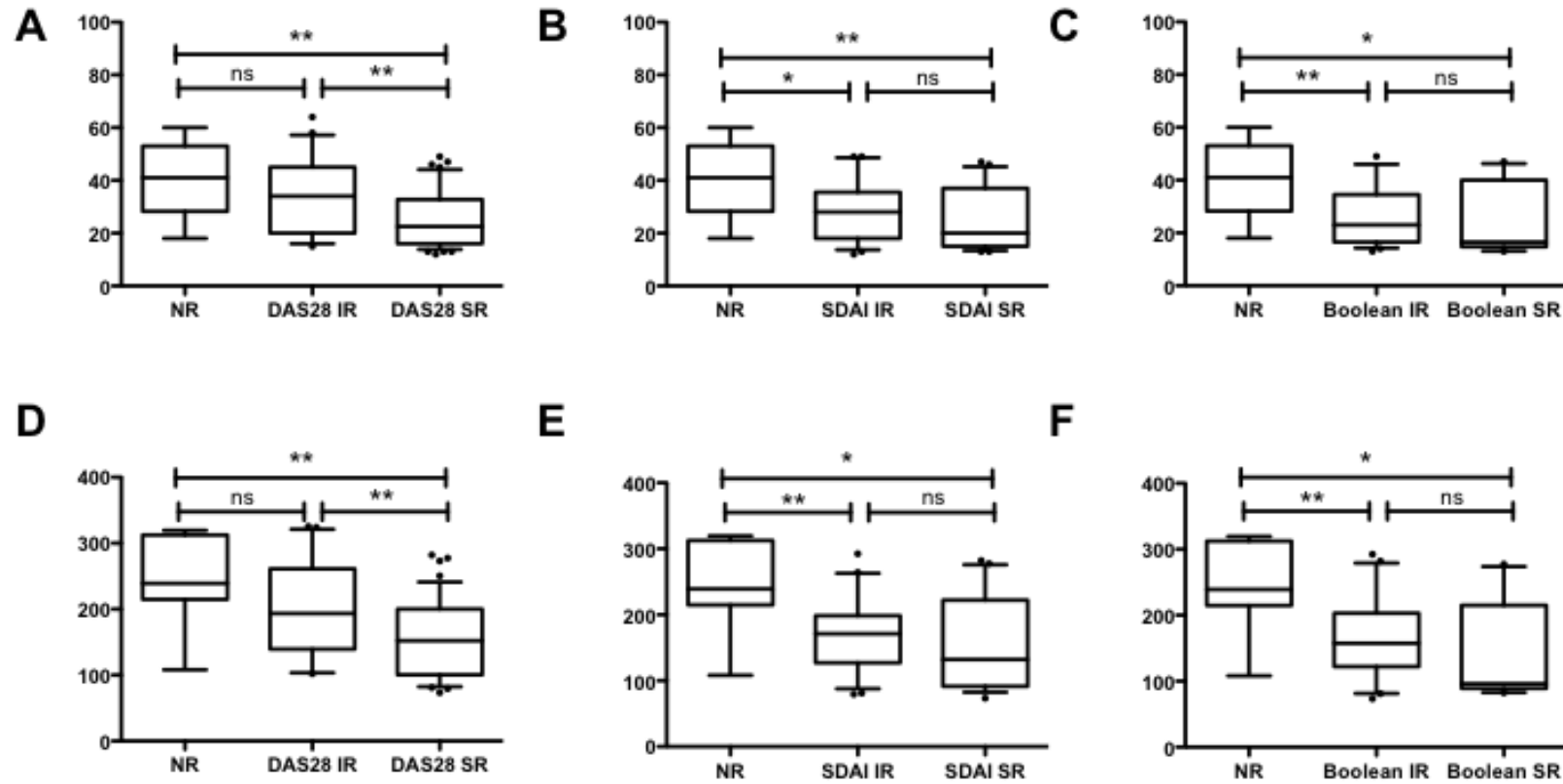


Figure 6-9 MBDA Scores in LDAS, IR and SR states

A-C = baseline MBDA, D-F = AUC MBDA, A&D = DAS28, B&E = SDAI, C&F = Boolean. Level of significance determined by Mann-Whitney U test. Values expressed as medians with IQR. NR = no remission by any criteria, IR = intermittent remission, SR = sustained remission. Levels of significance determined by Mann-Whitney test (NR vs IR, IR vs SR, LDAS vs SR). ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$

6.3.8 Serum biomarkers as predictors of sustained remission (over time)

Predictors of sustained DAS28ESR (Table 6.9)

Baseline calprotectin, CXCL10, hsCRP, leptin, SAA, TNFR1, VCAM1 and MBDA scores are predictors of sustained DAS28ESR remission (Standardised ORs range from 0.01 – 0.62). Time-integrated values of Calprotectin, CXCL10, hsCRP, Resistin, SAA, TNFR1, VCAM1 and MBDA score are also predictors (standardized ORs range from 0.13 – 0.58). Since all the standardized ORs are less than 1, it means that the lower the biomarker level, the more likely that the patient will be in sustained remission.

Predictors of sustained SDAI (Table 6.10)

Baseline Calprotectin, hsCRP, leptin, resistin, SAA and MBDA are predictors of sustained SDAI remission (Standardised ORs range 0.03 – 0.45). Calprotectin AUC, hsCRP AUC, Resistin AUC, SAA AUC and MBDA AUC are also predictors (Standardised ORs 0.11 – 0.48).

Predictors of sustained Boolean remission (Table 6.11)

Baseline MBDA score was the only predictor of sustained Boolean remission (Standardised OR 0.34). hsCRP AUC and MBDA AUC were the only time-integrated values which were predictors of sustained Boolean remission.

Table 6-9: Biomarker Predictors of Sustained DAS28 Remission
Univariate logistic regression assessing baseline serum biomarkers levels and time-integrated values of serum biomarkers over the first 6 months as predictors of sustained DAS28ESR remission over 1 year.

LDAS vs DAS28 SR	OR	Standardised OR	p value
Baseline Calprotectin	1 (1, 1)	0.42	0.004
Baseline CXCL10	1 (1, 1)	0.31	0.009
Baseline hsCRP	1 (1, 1)	0.38	0.004
Baseline IL6	1 (1, 1)	1.04	0.865
Baseline leptin	1 (1, 1)	0.54	0.012
Baseline SAA	1 (1, 1)	0.01	0.008
Baseline MBDA score	1 (1, 1)	0.36	<0.0001
Calprotectin AUC	1 (1, 1)	0.19	<0.0001
CXCL10 AUC	1 (1, 1)	0.48	0.006
HsCRP AUC	0.98 (0.96, 0.99)	0.39	0.005
IL6 AUC	1 (1, 1)	0.77	0.248
Leptin AUC	1 (1, 1)	1.23	0.635
SAA AUC	0.95 (0.92, 0.98)	0.13	0.003
MBDA AUC	1 (1, 1)	0.34	<0.0001

Table 6-10: Biomarker Predictors of Sustained SDAI Remission
Univariate logistic regression assessing baseline serum biomarkers levels and time-integrated values of serum biomarkers over the first 6 months as predictors of sustained SDAI remission over 1 year.

LDAS vs SDAI SR	OR	Standardised OR	p value
Baseline Calprotectin	1 (1, 1)	0.34	0.029
Baseline CXCL10	1 (1, 1)	0.25	0.076
Baseline hsCRP	1 (1, 1)	0.36	0.019
Baseline IL6	1 (1, 1)	0.97	0.932
Baseline leptin	1 (1, 1)	0.45	0.01
Baseline SAA	1 (1, 1)	0.03	0.008
Baseline MBDA score	0.91 (0.84,0.96)	0.28	0.007
Calprotectin AUC	1 (1, 1)	0.11	0.002
CXCL10 AUC	1 (1, 1)	0.60	0.205
hsCRP AUC	0.98 (0.96, 1)	0.48	0.042
IL6 AUC	1 (1, 1)	0.75	0.249
Leptin AUC	1 (1, 1)	1.11	0.838
SAA AUC	0.95 (0.92, 0.99)	0.15	0.008
MBDA AUC	0.98 (0.96, 0.99)	0.24	0.004

Table 6-11 Biomarker predictors of Sustained Boolean Remission
Univariate logistic regression assessing baseline serum biomarkers levels and time-integrated values of serum biomarkers over the first 6 months as predictors of sustained Boolean remission over 1 year.

LDAS vs Boolean SR	OR	Standardised OR	p value
Baseline Calprotectin	1 (1, 1)	0.42	0.214
Baseline CXCL10	1 (1, 1)	0.41	0.405
Baseline hsCRP	1 (1, 1)	0.03	0.057
Baseline IL6	0.99 (0.94, 1.04)	0.56	0.607
Baseline leptin	1 (1, 1)	0.08	0.17
Baseline SAA	1 (1, 1)	0.00	0.183
Baseline MBDA score	0.92 (0.85, 1.00)	0.34	0.044
Calprotectin AUC	1 (1, 1)	0.20	0.061
CXCL10 AUC	1 (1, 1)	0.38	0.239
HsCRP AUC	0.92 (0.84, 1.00)	0.03	0.044
IL6 AUC	1 (1, 1)	0.68	0.397
Leptin AUC	0.97 (0.92, 1.02)	0.00	0.194
SAA AUC	0.87 (0.75, 1.01)	0.00	0.06
MBDA AUC	0.98 (0.97, 1.00)	0.31	0.034

6.4 Discussion

Reliable assessment of remission is important for the optimal management of patients with RA. Imaging studies have shown that majority of patients in remission, whether the administered therapy was a DMARD or a biological agent, have some evidence of active subclinical inflammation. (Brown et al. 2006, Saleem et al. 2009). These findings indicate that patients in clinical remission have subclinical disease despite displaying few clinical signs and symptoms. This highlights the need for identifying new measures to supplement clinical assessment of remission status. The REMIRA study demonstrates a panel of novel biomarkers which could be used to address this. This cohort of patients, whilst being clinically similar in phenotype, demonstrates surprising heterogeneity at the molecular level. Despite this heterogeneity, we identified several individual biomarkers which were able to define remission states. MBDA and some of its analytes (hsCRP, SAA, IL6, Leptin), Calprotectin and CXCL10 were able to differentiate between LDAS and all 3 point remission criteria. In addition, TNFR1 and VCAM1 were able to differentiate between LDAS and DAS28ESR point remission. MBDA, hsCRP, SAA, IL6, leptin, CXCL10 at baseline or over 6 months were able to predict the frequency of remission over 12 months. Calprotectin over 6 months but not at baseline were able to predict the frequency of remission over 12 months indicating that under some circumstances, it maybe beneficial to capture multiple readings to inform outcome more accurately. It should also be emphasized that, since these analytes were measured in serum as opposed to synovium or synovial fluid, the data indicate that it is possible to discriminate between low disease activity states by measuring inflammatory responses at a systematic rather than local level.

The MBDA score and the individual biomarkers which have been shown to be of significance as a biomarker are discussed below:

6.4.1 MBDA score

A significant association between the MBDA score and DAS28-CRP has been previously shown in a heterogeneous group of RA patients with a wide range in autoantibody status, disease activity and RA treatment (Curtis et al. 2012). The MBDA score was able to consistently distinguish patients in different categories of clinical disease activity. The performance of the MBDA score was assessed using the AUROC previously for classifying patients into low versus moderate or high disease activity using DAS28CRP. This showed AUROC of 0.77 and 0.70 respectively (Curtis et al. 2012). No comparisons were made in remission. The REMIRA study shows that the baseline MBDA score interestingly preformed the least well with baseline DAS28CRP. The agreement of sustained MBDA remission with the sustained clinical remission is better over time than point remission. This suggests that a trend over time is more valuable to obtain the true picture of a clinical state than just a cross-sectionally snapshot. The baseline median MBDA score was below the remission threshold (<25) with all 3 point clinical remission and sustained remission. Further work is under way to determine whether this current MBDA set points for remission should be lowered.

6.4.2 Acute phase response biomarkers (hsCRP and SAA)

hsCRP and SAA have been shown in this study to be important biomarkers. They were all highly significanty different when comparing LDAS with all 3 point

remission criteria. Baseline levels and time-integrated values over 6 months were able to predict sustained remission over 1 year with all 3 remission criteria.

During active inflammation, synovial inflammation is mirrored by a systemic acute-phase response in which marked biosynthetic changes in the liver alter plasma protein concentrations (Steel, Whitehead 1994). Both SAA and CRP are key acute phase response markers. Serum amyloid A (SAA) proteins are a family of apolipoproteins. SAA mRNA is expressed in RA synovium and not in healthy controls (O'Hara et al. 2000). SAA exists as constitutive (c-SAA) and acute-phase isoforms (A-SAA). A-SAA induces angiogenesis, leukocyte recruitment, and chemokine and MMP expression in RA (Mullan et al. 2006). Similar to CRP, levels of SAA increase within hours after inflammatory stimulus, and the magnitude of increase may be greater than that of CRP. SAA levels correlate better with disease activity in early disease compared to ESR and CRP. This seems to be more specific in RA than other forms of inflammatory arthritis (Cunnane et al. 2000). It correlates with the 28-joint swollen joint count and was independently associated with 1-year radiographic progression (Connolly et al. 2012).

CRP correlates with disease activity, radiologic progression and treatment response (Nakamura 2000). Conventional CRP assays are not able to differentiate low levels of inflammation therefore the use of hsCRP assays is more valuable at low levels of disease activity. It correlated better with other disease activity measures when compared to ESR (Dessein, Joffe & Stanwix 2004). The mean hsCRP level of apparently healthy men is <2mg/L (Ridker et al. 1997). The median

hsCRP levels of patients in remission in this study were below this threshold with all 3 remission criteria suggesting that the levels normalise in remission.

Our study also showed that SAA performed better than hsCRP at predicting sustained DAS28 or SDAI remission. The significance for predicting Boolean remission was just over the >0.05 threshold.

6.4.3 IL-6

IL-6 is a pro-inflammatory cytokine that stimulates the hepatic synthesis of acute phase proteins. It has a central role in the pathogenesis of RA (Park, Pillinger 2007). IL-6 levels are elevated in RA patients compared to healthy controls (Knudsen et al. 2008) and multiple studies have shown the efficacy and safety of anti-IL6 blockade in the treatment of RA. (Schoels et al. 2013). Its role as a biomarker has been shown. It correlates not only with clinical variables of disease activity, such as joint counts and global disease activity scores, but also for the composite measures of disease activity (DAS, DAS-CRP, CDAI, and SDAI) (Milman, Karsh & Booth 2010). Several studies have demonstrated decreases in IL-6 levels in response to treatment including anti-TNF (Braun-Moscovici et al. 2006), Leflunomide (Litinsky et al. 2006) and Methotrexate (Crilly et al. 1995). The REMIRA study further supports its use as a biomarker in the low disease activity cohort. It performed well as a biomarker for differentiating between remission and LDAS as well as predicting sustained remission. This was not confirmed with logistic regression analysis.

6.4.4 Leptin

Leptin is a 16-kDa protein secreted by white adipose tissue and therefore is also an adipokine. It resembles IL-6 and is a member of the cytokine superfamily. It is present in the synovial fluid (Bokarewa et al. 2003). Higher levels have been found in RA patients compared to healthy controls in some studies (Bokarewa et al. 2003, Otero et al. 2006) but not in others (Hizmetli et al. 2007, Popa et al. 2005). Its use as a biomarker is still controversial. Studies have shown conflicting results: Anders et al., 1999 showed no difference between low and moderate disease activity, although the number of patients in each group were small (Anders et al. 1999). Leptin has also been shown to be inversely correlated with active chronic inflammation (Popa et al. 2005). Lee et al., 2007 showed higher serum leptin levels in RA patients with high disease activity, correlated well with disease activity, and decreased significantly when disease was well controlled (Lee et al. 2007). The REMIRA study is the first to demonstrate a role of leptin in defining remission amongst patients with low disease activity and furthermore, its role as a predictor of sustained remission. The strength of this study is the larger numbers of patients studied and also at multiple timepoints.

Leptin acts via the leptin receptor, which is a member of the IL6 receptor-related cytokine receptors. It exerts a pro-inflammatory effect on synovial fibroblasts (Tong et al. 2008) and chondrocytes (Gomez et al. 2011). The action of leptin in RA is not only targeted to articular tissue, it also exerts direct effects on activation, proliferation, maturation of, and production of inflammatory mediators by a variety of immune cells (Lam, Lu 2007). In particular, leptin is able to modulate regulatory T cells, which are potent suppressors of autoimmunity. Leptin sustains

Th1 immunity by promoting effector T cell proliferation and by constraining Treg cell expansion (De Rosa et al. 2007).

6.4.5 Calprotectin

Calprotectin is released from activated leucocytes during inflammation. Several studies have shown correlation between serum levels of calprotectin with ESR, CRP as well as swollen joint count (Brun et al. 1992, Andres Cerezo et al. 2011). Acute phase proteins are released from the hepatocytes during inflammation whereas calprotectin is released from the activated leucocytes which is derived from inflamed synovium. It therefore differs from acute phase proteins by directly reflecting the amount of activated leucocytes in the inflamed joints. There is evidence of a strong association between the decrease in calprotectin level and improvements in swollen joint counts (Brun et al. 1992, Andres Cerezo et al. 2011). This provides further evidence of its use as a suitable biomarker which gives information about the extent of local inflammation in affected joints. The use of calprotectin as a biomarker has been investigated in JIA patients who are in remission. It has been shown to have a role in predicting flares in patients after treatment withdrawal (Foell et al. 2010, Gerst et al. 2012). This biomarker informs about the activation status of innate immunity at the molecular level and thereby has a role in identifying patients who are in remission.

6.4.6 CXCL10

CXCL10 and its receptor CXCR3 play important roles in leukocyte homing to inflamed tissues and in the perpetuation of inflammation. High levels of serum levels of CXCL0 have been found in active RA and significantly reduced with

response to treatment (Kuan et al. 2010). The REMIRA study has also showed that lower levels of CXCL10 was associated with lower levels of disease activity.

6.4.7 Implications for clinical practice

In an era where treatments are increasingly targeted at a molecular level, it is important that monitoring treatment responses evolve to reflect such specific therapies. This study has demonstrated that novel laboratory biomarkers, beyond ESR, play important roles in defining remission states and predicting sustained remission.

This chapter has shown that low disease activity states are heterogeneous on a molecular level. It follows from this that more than 1 biomarker is likely to be required to accurately identify patients in remission. Several factors with different mechanisms of action have been identified in this study. Sustained remission was associated with lower baseline MBDA scores as well as lower concentrations of IL-6, SAA, hsCRP and leptin, indicating that presence of subclinical inflammation may play an important role even at low levels of disease activity. It was not possible to compare the different remission groups directly as the patients overlapped in these groups. However, it is apparent that levels of the leptin, IL-6, SAA, hsCRP and MBDA scores were lower with the more stringent criteria of remission at baseline and over time. This was not mirrored for CXCL10 and calprotectin levels.

The study also explored the use of serial serum measurements will have a role in the clinical setting. Having 3 readings over a 6 month period seems to have more

predictive power with some of the biomarkers. This suggests that stability of the molecular signature is important to predict clinical outcome.

In conclusion, even at the low end of the disease activity, the data indicate that the MBDA score and some of its analytes can differentiate between small changes in disease activity. These findings highlight the importance of close follow-up of patients who have achieved low disease activity or clinical remission and the potential value of repeated measurements and assessments to evaluate stability of phenotypes over time. It is proposed that molecular markers of inflammation may be included in the evaluation of patients with low disease activity states to facilitate therapy decisions such as drug tapering or treatment withdrawal.

7 Impact of remission on health related quality of life

7.1 Introduction

Pain, fatigue and disability are all characteristic features of RA. Over time the disability associated with RA can have considerable impacts on health-related quality of life (HRQoL) (Scott, Smith & Kingsley 2005, Scott et al. 2000). One important aim of treatment is to maximise HRQoL.

Several instruments have been used to assess HRQoL in RA patients. These include generic tools eg SF36 (Medical outcomes study 36-Item Health Survey forms), EuroQol and Functional Assessment of Chronic Illness Therapy (FACIT-F) as well as the disease-specific tool Health assessment questionnaire (HAQ). Although, it is well-established that RA therapies decrease disability (Ma, Kingsley & Scott 2010) and improve HRQoL (Kimel et al. 2008, Kosinski et al. 2002), the direct relationship between remission and HRQoL outcomes are less well established (Kekow et al. 2010). 1 study has reported on the benefit of remission over low disease activity states cross-sectionally (Radner, Smolen & Aletaha 2014), but the impact of sustained remission on HRQoL have not been reported in detail.

This study evaluated the REMIRA cohort to explore the relationship between baseline remission, sustained remission and HRQoL.

7.2 Methods and Patients

7.2.1 REMIRA cohort

This has been described previously in chapter 5.

7.2.2 Clinical assessments

Clinical assessments have been described previously in chapter 5.

Patient reported outcome measures

Health Assessment Questionnaire Disability Index (HAQ-DI) ranged from 0-3 with higher scores indicating more disability. The Medical Outcomes Study 36-Item Short-Form Health Survey (SF36) assesses health-related quality of life. There are 8 domains: physical function (PF), physical role (RP), bodily pain (BP), general health perception (GH), vitality (VT), social function (SF), emotional role (RE) and mental health (MH). These domains are then generated into two summary measures: the physical component score (PCS; including PF, RP, BP and GH) and the mental component score (MCS including VT, SF, RE and MH). Both range from 0-100. Norm-based scoring algorithms were used for the subscales, for which scores have a mean of 50 and a SD of 10. EuroQol also known as EuroQol is a standardized instrument for use as a measure of health outcome. There are 5 domains which can be combined to a total of 243 health states. Each health state can be associated with a numeric score on which full health has a value of 1, death has a value of 0 and unconscious is -0.402. In addition, the EuroQol VAS which is a 20cm vertical VAS that generates a self-rating of health-related quality of life. The Functional Assessment of Chronic Illness Therapy (FACIT-F, (Yellen et al. 1997)) is a 13 item questionnaire that assesses self-reported fatigue (range 0-52). With

SF36, EurQol and FACIT-F, the greater the score, the better the outcome. These outcomes were all measured 3-monthly for the length of the study.

7.2.3 Statistical analysis

STATA 11.2 (StataCorp, College Station, TX, USA) was used for statistical analysis. Point remission at baseline is defined as DAS28ESR <2.6, DAS28CRP <2.32, SDAI \leq 3.3 and CDAI \leq 2.8. ACR/EULAR Boolean remission was defined as TJC, SJC, CRP (mg/dl) and patient VAS (0-10cm) all \leq 1. Sustained remission (SR) is defined as achieving remission at all visit time-points, intermittent remission (IR) is defined as achieving remission in at least 1 visit time-point but not all. No remission (NR) is defined as not achieving remission at any visit time-point. Individual variables were assessed descriptively as median values and interquartile ranges (IQR).

To assess the impact of remission on HRQoL over 1 year, time-integrated values were calculated using area under the curve (AUC). These were computed using GraphPad Prism software using the trapezoidal method. Last observations carried forward method was used to handle missing data. Comparisons of these HRQoL measures between remission vs non-remission at baseline and between NR vs IR, IR vs SR and NR vs SR were performed using Mann-Whitney test. Generalised estimating equation analysis (GEE) was used to estimate the effect of different remission groups over the timepoints during 12 months of follow-up. This analysis was limited to DAS28 as the number of patients in the other 2 sustained remission criteria were too small for meaningful analysis.

7.3 Results

7.3.1 Impact Of Initial Point Remission Status On HRQoL

Patients meeting one of the three remission criteria at baseline (DAS28ESR, SDAI or Boolean) were compared with patients in LDAS states. With all remission criteria patients had significantly better baseline FACIT-F, SF36, HAQ and Euroqol scores if they were in remission compared to patients in LDAS states (Table 7.1)

Patients in remission at baseline continued to have significantly better outcomes in all HRQoL outcomes measured over the ensuing 12 months. This was shown by comparing AUCs for HRQoL in patients in initial remission with LDAS patients. This finding was consistent across all 3 remission criteria (Table 7.1); it included HAQ, EuroQol, EuroQol VAS and SF36.

Table 7-1 Baseline and AUC HRQoL between LDAS and Baseline Remission

Comparing low disease activity state at baseline (LDAS) with baseline DAS28ESR, SDAI and Boolean remission

*All values are reported as median (IQR). * Mann-Whitney test. HRQoL = Health related quality of life, FACIT –F = Functional assessment of chronic illness therapy – Fatigue, SF36 = short form 36, PCS = physical component score, MCS = mental component score, HAQ = health assessment questionnaire, AUC = area under the curve.*

HRQoL	LDAS	DAS28 Remission	P Value*	SDAI Remission	P Value*	Boolean Remission	P Value*
<i>FACIT-F</i>	35 (31, 38)	43 (38, 47)	<0.0001	46 (42, 50)	<0.0001	46 (43, 50)	<0.0001
<i>SF36 PCS</i>	39 (34, 43)	48 (40, 53)	<0.0001	51 (46, 55)	0.001	52 (48, 55)	<0.0001
<i>SF36 MCS</i>	49 (41, 53)	54 (47, 58)	0.021	57 (51, 58)	<0.0001	57 (51, 58)	0.002
<i>HAQ</i>	0.75 (0.5,1.38)	0 (0, 0.5)	<0.0001	0 (0, 0.125)	<0.0001	0 (0, 0.125)	<0.0001
<i>Euroquol</i>	0.69 (0.59,0.76)	0.80 (0.69, 1.00)	0.024	1.00 (0.80, 1.00)	<0.0001	1.00 (0.80, 1.00)	<0.0001
<i>Euroqol VAS</i>	70 (60, 80)	81 (75, 93)	0.002	90 (80, 95)	<0.0001	90 (80, 95)	<0.0001
<i>FACIT-F AUC</i>	414 (349, 488)	537 (455, 575)	<0.0001	553 (492, 589)	<0.0001	569 (488, 594)	0.001
<i>SF36 PCS AUC</i>	467 (430, 512)	588 (513, 638)	<0.0001	622 (554, 695)	<0.0001	622 (585, 655)	<0.0001
<i>SF36 MCS AUC</i>	552 (500, 648)	618 (557, 674)	0.005	649 (583, 691)	0.001	663 (597, 689)	0.007
<i>HAQ AUC</i>	10.3 (4.0,14.1)	1.2 (0, 6.6)	<0.0001	0.3 (0, 1.8)	<0.0001	0.43 (0, 1.88)	<0.0001
<i>Euroquol AUC</i>	8.7 (7.7, 9.3)	9.9 (8.7, 11.4)	<0.0001	10.6 (9.5,11.7)	<0.0001	11.1 (9.6, 12.0)	<0.0001

Changes in outcome measures over time for patients meeting different point remission criteria compared to LDAS are summarized in Tables 7.2-7.6.

When assessing the outcomes at each time point over the 12 month period of study, the median HAQ within the LDAS group ranged from 0.75 to 1, whereas the median HAQ was significantly lower in the DAS28, SDAI and Boolean point remission groups at each time point (0-0.125, 0 and 0 respectively, Table 7.2). The median EuroQol in the LDAS group ranged from 0.69 - 0.76. These were lower than DAS28ESR (0.80 - 0.81), SDAI (0.85 - 1) and Boolean (0.94 - 1) point remission groups at all time points (Table 7.3). The median FACIT-F was also significantly higher at all time points in DAS28 (44-47), SDAI (46-48) and Boolean (47-49) point remission groups compared to LDAS group (34-36) (Table 7.4).

The median SF36 PCS of the LDAS group was 38.48 - 41.47. This was significantly lower at all time points than DAS28 remission (47.78 - 49.40), SDAI (50.90 - 52.95) and Boolean (52.04 - 53) (Table 7.5). The median SF36 MCS in the LDAS group was 45.36 - 50.08. This was lower significantly lower only at T0 and T12 in the DAS28 groups (53.54 - 54.06). The median SF36 MCS in the SDAI group was 54.55-57 and 55.04-56.67 in the Boolean group. In both these groups, these were not significantly different at T6 compared to the LDAS group (Table 7.6).

Although it was not possible to compare between the remission groups, there is a trend of greater improvement with increasing stringency of the remission criteria.

Table 7-2 HAQ Scores At Each Time Point By LDAS and Remission Groups

Shows median scores (IQR) of patients in LDAS group and three remission groups - DAS28, SDAI and Boolean remission.

Time point	LDAS	DAS28	SDAI	Boolean
0	0.75 (0.50, 1.38)	0 (0, 0.50)*	0 (0, 0.13)*	0 (0, 0.13)*
3	0.75 (0.25, 1.06)	0 (0, 0.63)*	0 (0, 0.13)*	0 (0, 0.13)*
6	0.81 (0.31, 1.13)	0.06 (0, 0.56)*	0 (0, 0.13)*	0 (0, 0.13)*
9	0.94 (0.38, 1.50)	0.13 (0, 0.38)*	0 (0, 0.13)*	0 (0, 0.25)*
12	1.00 (0.13, 1.25)	0 (0, 0.25)*	0 (0, 0.13)*	0 (0, 0.13)*

p values * = <0.0001, + = <0.001, ‡ = <0.01, # = <0.05

Table 7-3 EuroQol Scores At Each Time Point By LDAS and Remission Groups

Shows median scores (IQR) of patients in LDAS group and three remission groups - DAS28, SDAI and Boolean remission.

Time point	LDAS	DAS28	SDAI	Boolean
0	0.73 (0.65, 0.78)	0.80 (0.71, 1.00)+	0.93 (0.80, 1.00)*	1.00 (0.80, 1.00)*
3	0.76 (0.69, 0.78)	0.81 (0.69, 1.00)+	1.00 (0.80, 1.00)*	1.00 (0.80, 1.00)*
6	0.76 (0.69, 0.78)	0.80 (0.73, 1.00)#	0.88 (0.80, 1.00)*	0.94 (0.80, 1.00)*
9	0.73 (0.59, 0.80)	0.80 (0.73, 1.00)+	0.85 (0.80, 1.00)*	1.00 (0.80, 1.00)*
12	0.69 (0.59, 0.80)	0.80 (0.74, 1.00)*	1.00 (0.76, 1.00)*	1.00 (0.80, 1.00)*

* = <0.0001, + = <0.001, ‡ = <0.01, # = <0.05, ns = non-significant

Table 7-4 FACIT-F Scores At Each Time Point By LDAS and Remission Groups

Shows median scores (IQR) of patients in LDAS group and three remission groups - DAS28, SDAI and Boolean remission.

Time point	LDAS	DAS28	SDAI	Boolean
0	35 (31, 41)	44 (38, 48)*	46 (42, 50)*	47 (43, 50)*
3	35 (29, 42)	44 (39, 48)*	47 (41, 49)*	48 (42, 49)*
6	36 (29, 41)	47 (41, 49)*	48 (45, 50)*	48 (46, 50)*
9	34 (26, 41)	44 (38, 49)*	47 (42, 50)*	49 (42, 51)*
12	34 (26, 38)	45 (39, 49)*	47 (42, 50)*	48 (43, 50)*

* = <0.0001, + = <0.001, ‡ = <0.01, # = <0.05

Table 7-5 SF36 PCS Scores At Each Time Point By LDAS and Remission Groups

Shows median scores (IQR) of patients in LDAS group and three remission groups - DAS28, SDAI and Boolean remission.

Time point	LDAS	DAS28	SDAI	Boolean
0	39.1 (34.1, 43.2)	48.4 (41.1, 53.8)*	51.5 (45.7, 54.8)*	52.3 (47.6, 55.2)*
3	41.5 (35.8, 44.5)	49.4 (40.1, 53.9)+	52.9 (46.3, 55.7)*	53.0 (47.6, 56.0)*
6	40.1 (32.4, 43.5)	48.6 (42.9, 54.7)*	50.9 (46.9, 54.9)*	52.0 (48.5, 56.3)*
9	38.9 (34.7, 42.0)	47.8 (40.9, 53.3)*	51.7 (45.7, 54.7)*	52.3 (47.1, 54.7)*
12	38.5 (31.3, 43.7)	49.2 (43.2, 53.5)*	51.7 (44.8, 55.4)*	52.2 (44.8, 55.4)*

* = <0.0001, + = <0.001, ‡ = <0.01, # = <0.05

Table 7-6 SF36 MCS Scores At Each Time Point By LDAS and Remission Groups

Shows median scores (IQR) of patients in LDAS group and three remission groups - DAS28, SDAI and Boolean remission.

Time point	LDAS	DAS28	SDAI	Boolean
0	48.9 (41.2, 53.5)	54.1 (47.0, 58.1)#	56.67 (50.5, 58.4)‡	56.7 (50.8, 58.5)‡
3	46.8 (40.9, 52.5)	53.7 (45.2, 57.3)ns	54.8 (46.7, 57.6)#	56.3 (49.0, 57.6)‡
6	50.1 (42.9, 57.5)	53.7 (47.0, 57.2)ns	54.5 (48.0, 57.9)ns	55.0 (49.7, 58.1)ns
9	47.0 (37.4, 54.6)	52.8 (42.9, 56.3)ns	54.6 (50.1, 57.7)‡	55.8 (49.7, 58.4)‡
12	45.4 (35.9, 51.4)	53.5 (42.1, 57.6)#	55.6 (50.6, 58.8)+	55.6 (51.5, 58.8)‡

*= <0.0001, + = <0.001, ‡ = <0.01, # = <0.05, ns= non-significant

7.3.2 Impact of frequency of remission on HRQoL

Table 7.7-7.11 summarised the levels of HRQoL outcomes achieved at each visit in no remission (NR), intermittent remission (IR) and sustained remission (SR) groups. They reveal relative stability of these values over time.

The NR group had higher median HAQ (0.875 – 1.5) than DAS28 IR (0.25 – 0.5) and DAS28 SR (0- 0.063). This trend was also observed in SDAI IR (0 – 0.25) and SDAI SR (0). Boolean IR and Boolean SR were similar (0 vs 0 – 0.125) (Table 7.7). Patients in the NR group lower median EuroQol values (0.52-0.66) compared to all 3 IR groups (DAS28 IR = 0.76 – 0.8, SDAI IR = 0.80, Boolean IR = 0.85-0.88). All SR groups achieved the highest EuroQol (DAS28 SR = 0.80 – 1, SDAI SR = 1, Boolean = SR 1) (Table 7.8). Over time, the NR patients achieved lower median FACIT-F values (26-33) compared to all 3 IR groups (DAS28 IR = 38-41, SDAI IR = 46-49, Boolean IR = 45-48). All SR remission groups achieved the highest FACIT-F (DAS28 SR = 45-47, SDAI SR = 46-49, Boolean SR = 47-50) (Table 7.9). Over time, patients in NR group had lower median SF36 PCS values (36.73 – 40.83) compared to all 3 IR groups (DAS28 IR = 31.56 – 43.83, SDAI IR = 44.02 – 47.02, Boolean IR = 50.08 – 51.98). All SR remission groups achieved the highest SF36 PCS (DAS28 SR = 49.37 – 52.60, SDAI SR = 51.05 – 54.56, Boolean SR = 52.04 – 56.31) (Table 7.10). Over time, NR patients achieved lower median SF36 MCS values (39.66 – 46.55) compared to all 3 IR groups (DAS28 IR = 48.29 – 51.90, SDAI IR = 50.72- 54.43, Boolean IR = 53.34 – 56.85). DAS28 SR and SDAI SR groups achieved the better SF36 MCS than IR and NR groups (DAS28 SR =

54.18 – 55.86 and SDAI SR = 55 – 57.23), Boolean SR was similar to Boolean IR (54.45 - 57) (Table 7.11).

When this was combined into time-integrated AUC values, there was a significant improvement of all HRQoL between NR, DAS28 IR and DAS28 SR and between LDAS, SDAI IR and SDAI SR (Fig 7.1-7.2).

Applying the most stringent Boolean remission criteria, the number of patients achieving sustained remission was small (10%). In all HRQoL measures, there were significant differences between Boolean NR vs Boolean IR. Interestingly, differences between Boolean IR and Boolean SR were not demonstrated in any of the outcomes (Fig 7.3).

The spidergrams in Figure 7.4 show the distribution of improvement in the AUC of the 8 domains of SF36. With DAS28 ESR and SDAI remission criteria, there were improvements in all 8 domains with increasing frequency of remission except in the GH domain. (Fig 7.4 A and B). There was no difference in the GH domain over time between NR and IR (Fig 7.4B). There were no improvements between Boolean IR and Boolean SR in all 8 domains (Fig 7C).

GEE analysis showed significant improvement of FACIT-F, EuroQol, SF36PCS and SF36 MCS between NR and DAS28 SR after correcting for baseline values. There was no difference in HAQ (Table 7.7).

Table 7-7 HAQ Scores At Each Time Point For NR, IR and SR Groups
Shows scores in no remission (NR), intermittent remission(IR) and sustained remission(SR) in three remission groups (DAS28, SDAI and Boolean remission) at each visit timepoint. Median scores and interquartile ranges are shown

Visit	NR	DAS28 IR	DAS28 SR	SDAI IR	SDAI SR	Boolean IR	Boolean SR
0	1.25 (0.75, 1.88)	0.50 (0, 0.94)	0 (0, 0.38)	0 (0, 0.63)	0 (0, 0.13)	0 (0, 0.25)	0 (0, 0.13)
3	0.88 (0.75, 1.44)	0.44 (0, 0.88)	0 (0, 0.38)	0.13 (0, 0.75)	0 (0, 0.25)	0 (0, 0.25)	0.06 (0, 0.44)
6	1.19 (0.69, 1.56)	0.38 (0, 0.88)	0.06 (0, 0.44)	0.13 (0, 0.75)	0 (0, 0.13)	0 (0, 0.13)	0.13 (0, 0.25)
9	1.50 (0.75, 1.63)	0.38 (0.13, 1.00)	0.06 (0, 0.31)	0.25 (0, 0.88)	0 (0, 0.13)	0 (0, 0.38)	0 (0, 0.25)
12	1.38 (0.50, 1.63)	0.25 (0, 1.13)	0 (0, 0.25)	0.13 (0, 0.75)	0 (0, 0)	0 (0, 0.13)	0 (0, 0.13)

Table 7-8 EuroQol Scores At Each Time Point For NR, IR and SR Groups
Shows scores in no remission (NR), intermittent remission(IR) and sustained remission(SR) in three remission groups (DAS28, SDAI and Boolean remission) at each visit timepoint. Median scores and interquartile ranges are shown

Visit	NR	DAS28 IR	DAS28 SR	SDAI IR	SDAI SR	Boolean IR	Boolean SR
0	0.66 (0.59 ,0.76)	0.76 (0.69,0.80)	0.80 (0.73, 1.00)	0.80 (0.73, 1.00)	1.00 (0.80, 1.00)	0.85 (0.80, 1.00)	1.00 (0.80, 1.00)
3	0.74 (0.60 ,0.78)	0.76 (0.69, 0.80)	1.00 (0.76, 1.00)	0.80 (0.73, 1.00)	1.00 (0.81, 1.00)	1.00 (0.80, 1.00)	1.00 (0.81, 1.00)
6	0.69 (0.55, 0.76)	0.80 (0.73 ,0.80)	0.85 (0.73, 1.00)	0.80 (0.69, 1.00)	1.00 (0.80, 1.00)	0.88 (0.80, 1.00)	1.00 (0.71, 1.00)
9	0.69 (0.52 ,0.73)	0.76 (0.66, 0.80)	0.81 (0.80, 1.00)	0.80 (0.73, 1.00)	1.00 (0.80, 1.00)	0.85 (0.80, 1.00)	1.00 (1.00,1.00)
12	0.52 (0.52, 0.69)	0.76 (0.69, 0.80)	0.85 (0.76, 1.00)	0.80 (0.73, 1.00)	1.00 (0.85, 1.00)	0.85 (0.76, 1.00)	1.00 (1.00,1.00)

Table 7-9 FACIT-F Scores At Each Time Point For NR, IR and SR Groups

Shows scores in no remission (NR), intermittent remission(IR) and sustained remission(SR) in three remission groups (DAS28, SDAI and Boolean remission) at each visit timepoint. Median scores and interquartile ranges are shown

Visit	NR	DAS28 IR	DAS28 SR	SDAI IR	SDAI SR	Boolean IR	Boolean SR
0	33 (18, 37)	39 (33, 46)	45 (40, 48)*	43 (38, 48)	46 (43, 50)*	45 (42, 50)	47 (45, 50)‡
3	30 (25, 37)	40 (32, 45)	45 (40, 49)+	43 (38, 48)	48 (41, 49)+	48 (42, 49)	47 (41, 51)‡
6	32 (24, 42)	41 (35, 47)	47 (42, 49)‡	45 (39, 49)	48 (46, 50)‡	48 (45, 50)	48 (46, 51)#
9	28 (19, 34)	40 (31, 46)	45 (39, 50)+	45 (38, 49)	49 (43, 50)+	47 (41, 50)	49 (46, 51)+
12	26 (19, 36)	38 (33, 45)	47 (41, 50)+	45 (37, 47)	49 (47, 51)*	47 (41, 50)	50 (47, 50)+

Table 7-10 SF36 PCS Scores At Each Time Point For NR, IR and SR Groups

Shows scores in no remission (NR), intermittent remission(IR) and sustained remission(SR) in three remission groups (DAS28, SDAI and Boolean remission) at each visit timepoint. Median scores and interquartile ranges are shown

Visit	NR	DAS28 IR	DAS28 SR	SDAI IR	SDAI SR	Boolean IR	Boolean SR
0	36.7 (32.8, 44.4)	43.1 (36.8, 48.0)	49.5 (41.2, 54.2)	47.0 (40.5, 52.4)	51.1 (44.4, 54.8)	52.0 (45.6, 54.5)	52.0 (49.3, 55.2)
3	39.8 (35.3, 45.2)	43.8 (35.0, 48.4)	52.6 (40.7, 55.1)	44.9 (39.6, 52.7)	53.0 (49.6, 55.5)	52.8 (47.1, 55.5)	53.0 (45.9, 56.0)
6	40.8 (34.6, 44.8)	43.3 (38.4, 47.6)	49.4 (43.0, 54.9)	46.9 (40.7, 50.1)	54.6 (49.4, 56.4)	50.1 (47.0, 54.7)	56.3 (45.6, 58.9)
9	34.7 (34.1, 35.9)	41.6 (38.4, 45.4)	48.7 (43.2, 53.8)	44.0 (40.3, 52.6)	52.4 (47.8, 56.6)	50.3 (46.2, 53.9)	53.9 (52.4, 57.2)
12	36.7 (32.4, 38.5)	41.7 (35.1, 47.6)	50.6 (43.9, 55.5)	45.5 (39.3, 51.4)	54.3 (49.2, 56.7)	51.4 (44.8, 55.0)	56.7 (49.2, 57.6)

Table 7-11 SF36 MCS Scores At Each Time Point For NR, IR and SR Groups
Shows scores in no remission (NR), intermittent remission(IR) and sustained remission(SR) in three remission groups (DAS28, SDAI and Boolean remission) at each visit timepoint. Median scores and interquartile ranges are shown

Visit	NR	DAS28 IR	DAS28 SR	SDAI IR	SDAI SR	Boolean IR	Boolean SR
0	46.6 (38.1, 53.2)	50.8 (44.3, 57.4)	55.9 (48.1, 58.2)	53.3 (46.3, 57.5)	57.2 (50.8, 58.5)	56.9 (48.4, 58.2)	55.3 (50.8, 58.1)
3	42.8 (31.4, 44.7)	51.3 (45.2, 57.5)	54.5 (45.4, 57.5)	52.5 (45.5, 56.7)	57.1 (51.1, 58.4)	54.8 (46.2, 58.4)	56.7 (51.6, 58.5)
6	42.3 (32.8, 51.9)	51.9 (47.2, 57.2)	54.4 (49.7, 57.8)	53.7 (48.0, 57.2)	57.1 (49.7, 58.3)	55.0 (49.7, 57.8)	54.5 (45.9, 58.1)
9	40.5 (32.5, 46.7)	48.3 (42.0, 54.6)	54.2 (50.0, 57.1)	51.1 (44.9, 55.6)	56.2 (51.9, 58.8)	53.5 (48.6, 57.4)	56.2 (52.7, 58.1)
12	39.7 (34.3, 48.5)	48.7 (40.8, 57.1)	54.9 (43.4, 58.5)	50.7 (43.2, 57.4)	55.0 (53.3, 58.8)	53.3 (42.1, 58.6)	57.0 (54.9, 58.8)

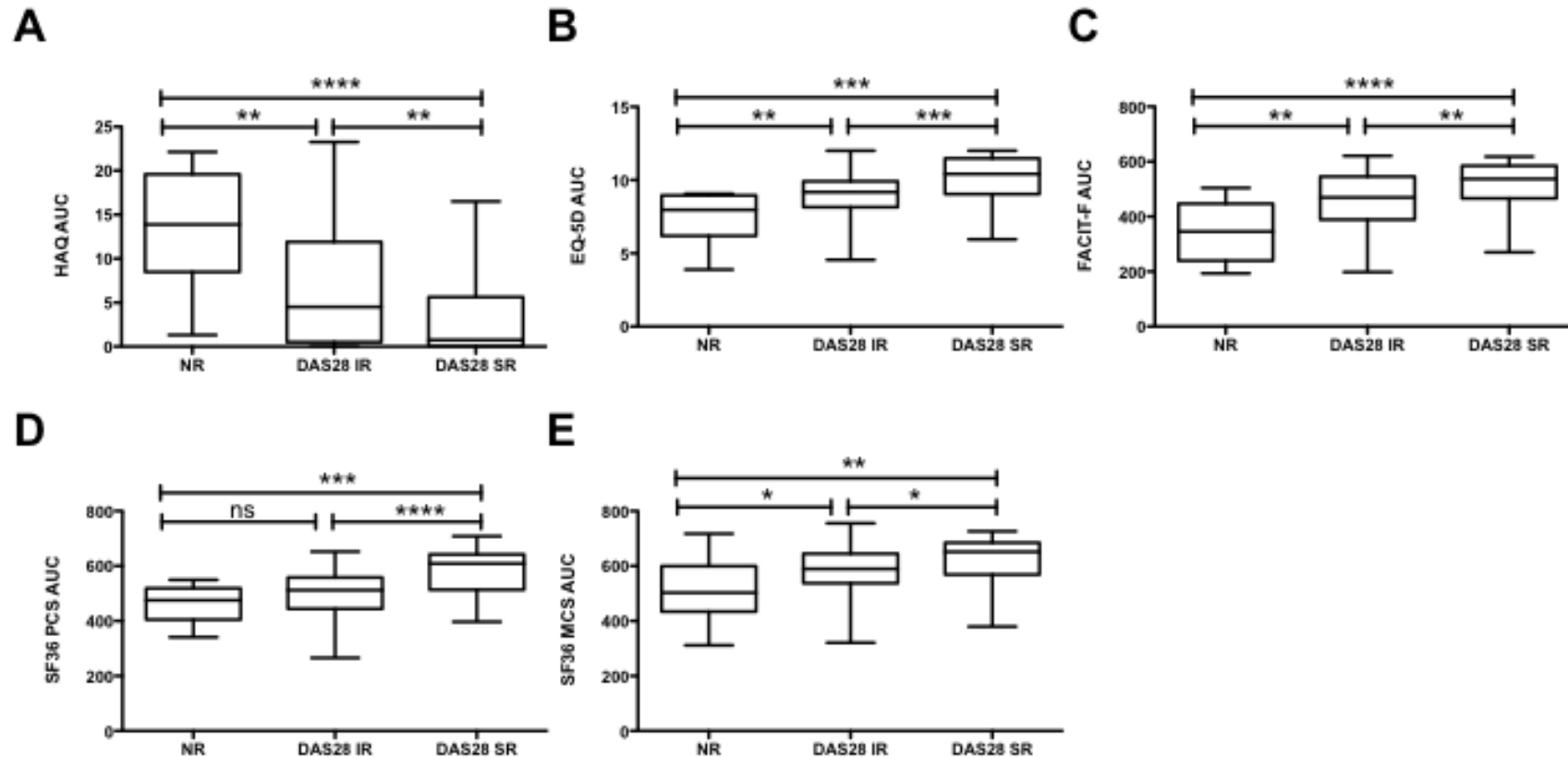


Figure 7-1 Impact of NR, DAS28 IR and SR on HRQoL over time

A) HAQ, B) EuroQol, C) FACIT-F, D) SF36 PCS, E) SF36 MCS, Values expressed as medians with min and max range. NR = No Remission by any criteria over 1 year), IR = intermittent remission, SR = sustained remission, ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$ using Mann-Whitney test (NR vs IR, IR vs SR, NR vs SR)

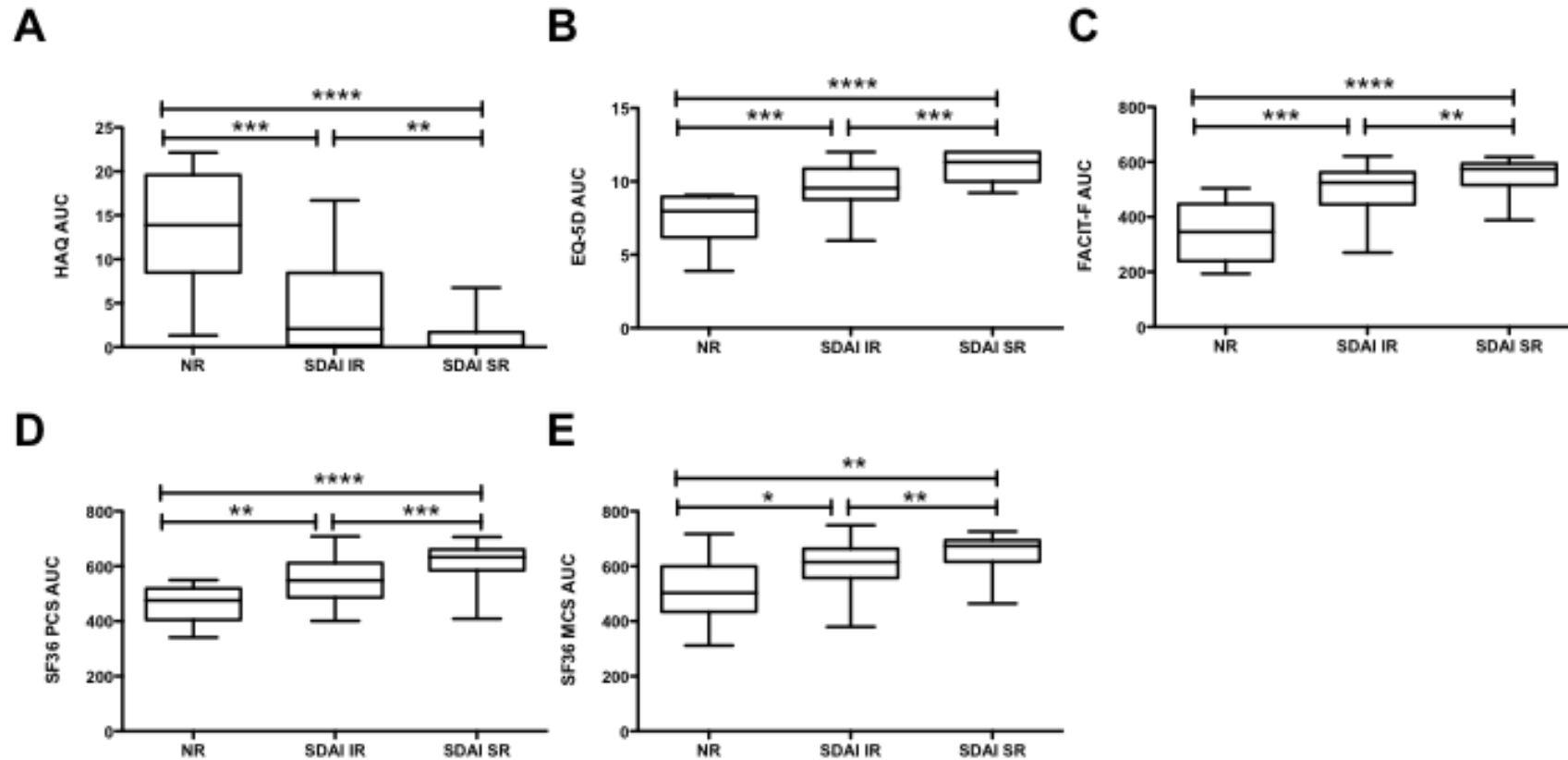


Figure 7-2 Impact of NR, SDAI IR and SR on HRQoL

A) HAQ, B) EuroQol, C) FACIT-F, D) SF36 PCS, E) SF36 MCS, Values expressed as medians with min and max range. NR = No Remission by any criteria over 1 year), IR = intermittent remission, SR = sustained remission, ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$ using Mann-Whitney test (NR vs IR, IR vs SR, NR vs SR)

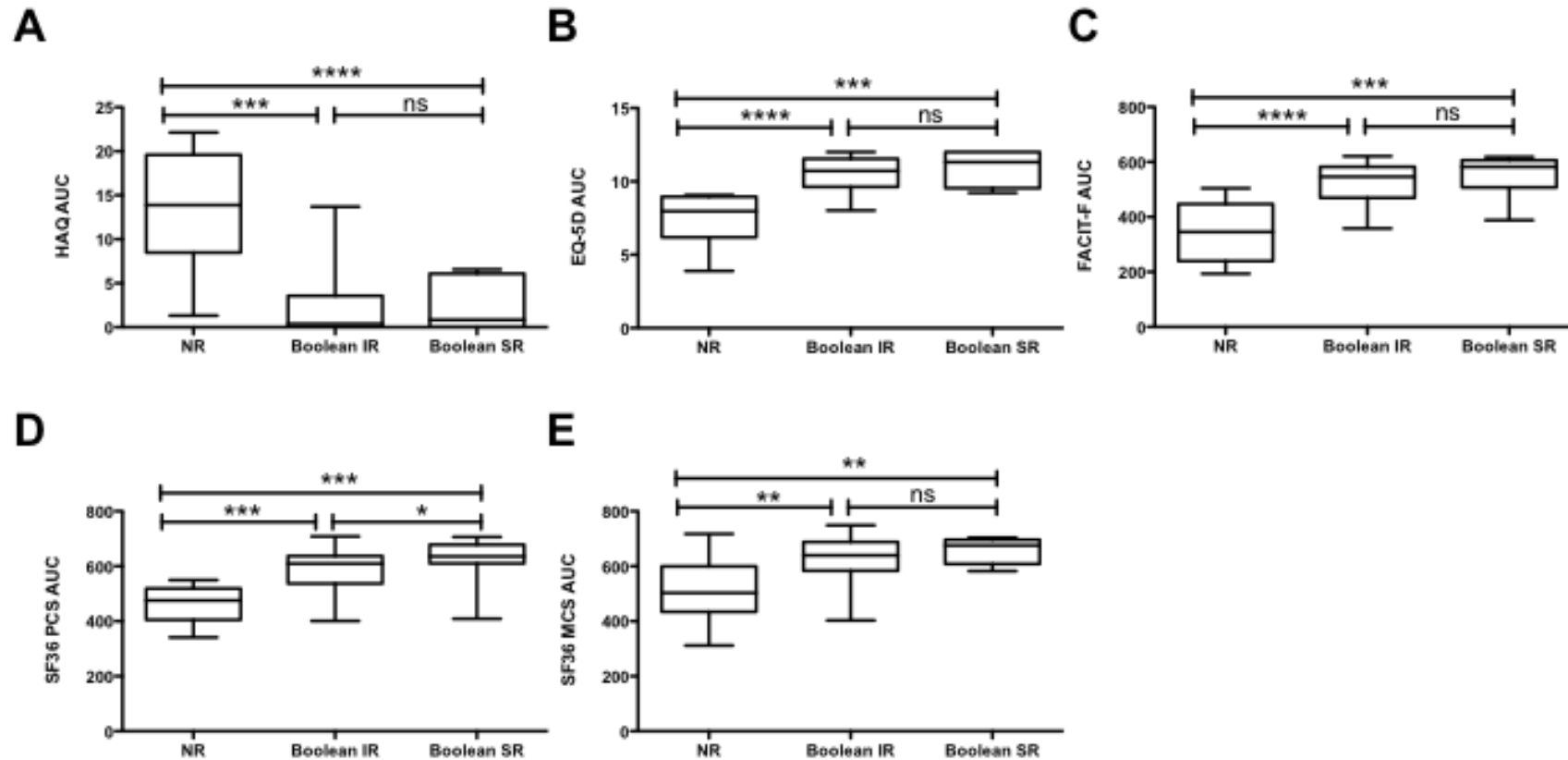


Figure 7-3 Impact of NR, Boolean IR and SR on HRQoL

A) HAQ, B) EuroQol, C) FACIT-F, D) SF36 MCS, E) SF36 PCS, Values expressed as medians with min and max range. NR = No Remission by any criteria over 1 year), IR = intermittent remission, SR = sustained remission, ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$ using Mann-Whitney test (NR vs IR, IR vs SR, NR vs SR)

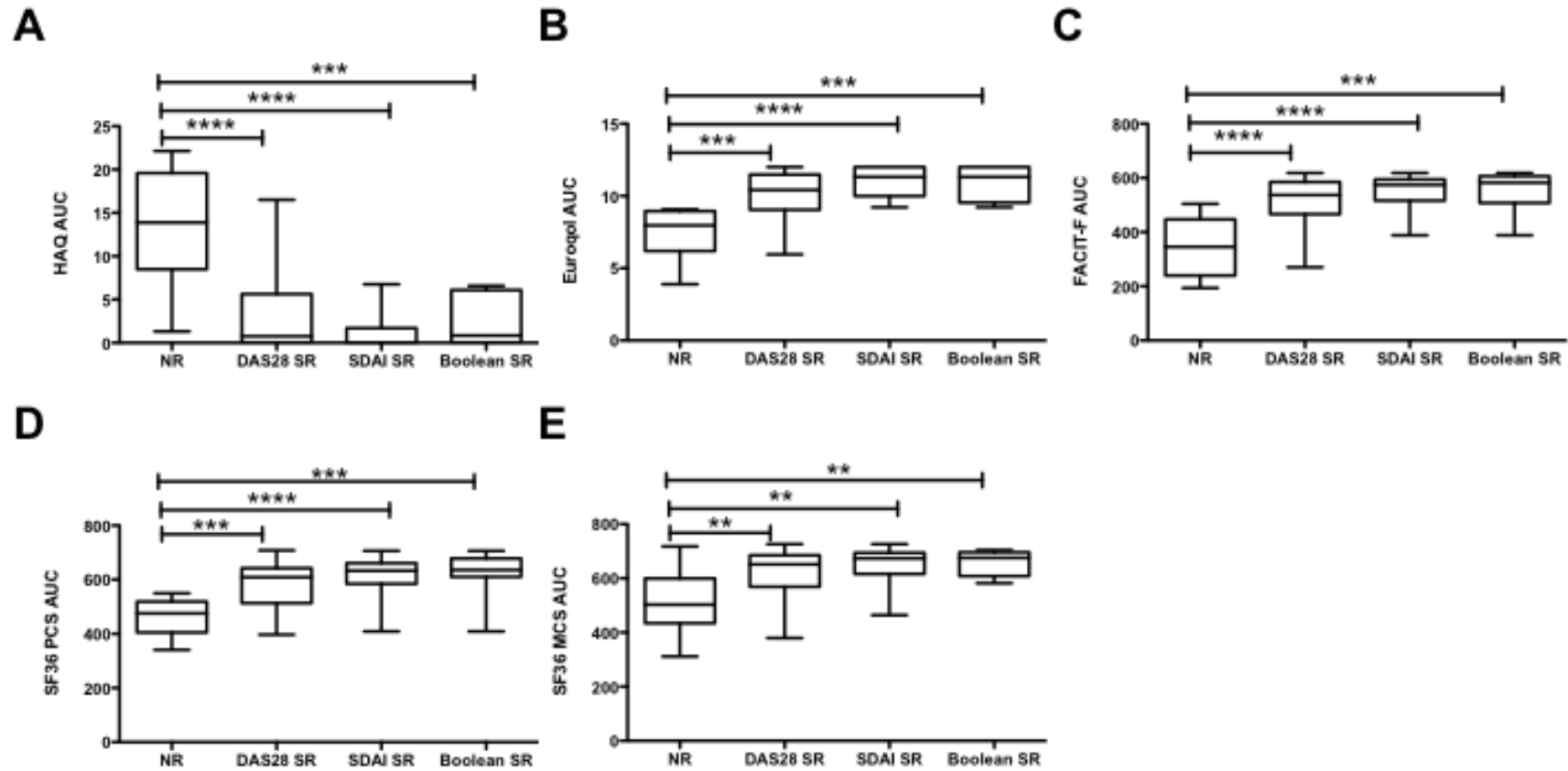


Figure 7-4. Impact of SR Compared With NR On HRQoL

A) HAQ, B) EuroQol, C) FACIT-F, D) SF36 PCS, E) SF36 MCS, Values expressed as medians with min and max range. NR = No Remission by any criteria over 1 year), IR = intermittent remission, SR = sustained remission, ns = $P > 0.05$, * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$, **** = $P \leq 0.0001$ using Mann-Whitney test (NR vs IR, IR vs SR, NR vs SR)

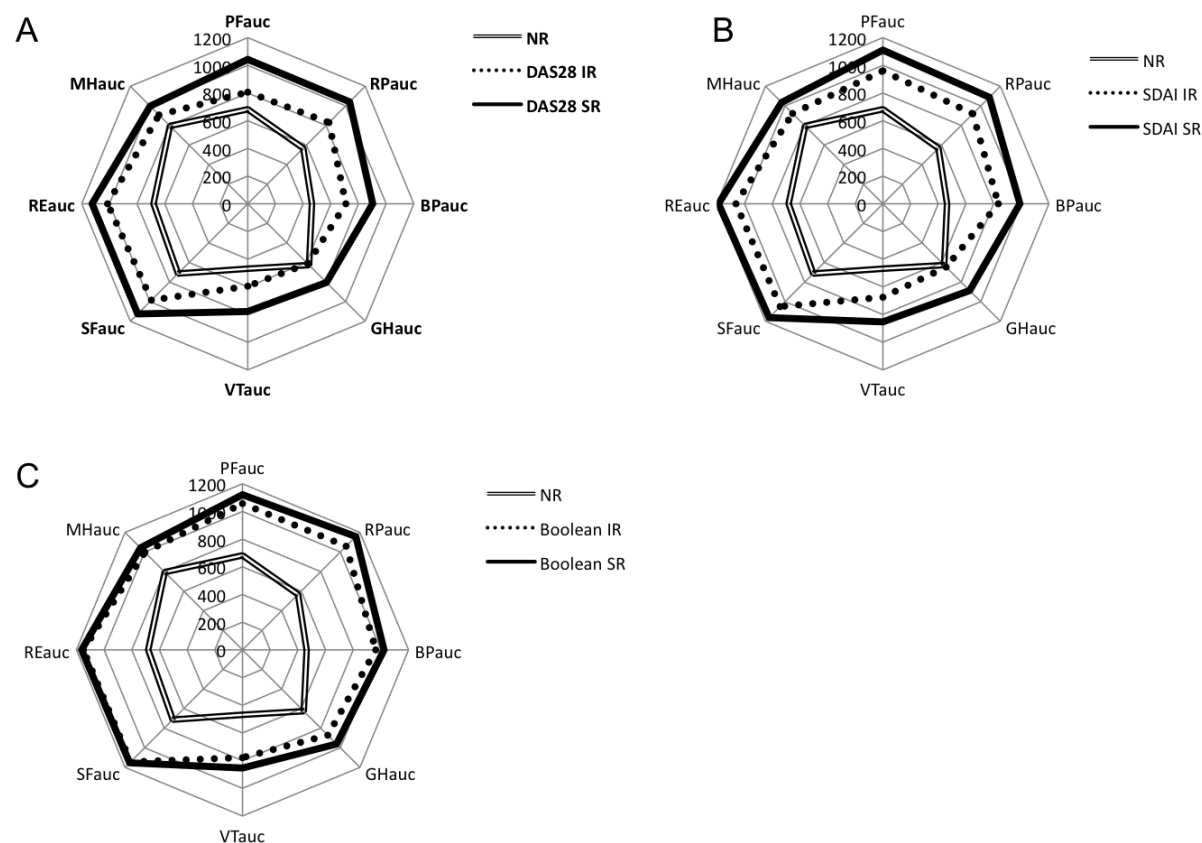


Figure 7-5 Spidergrams Showing 8 domains of SF36 in NR, IR and SR

A) DAS28 remission, B) SDAI remission, C) Boolean Remission, PR = physical functioning, RP = Role-physical, BP = bodily pain, GH = general health, VT = vitality, SF = social functioning, RE = Role-emotional, MH = mental health, values expressed as median AUC values

Table 7-12 GEE analysis comparison Of HRQoL of no remission and SR
Comparing no remission (NR) and different sustained remission groups (SR), GEE = General Estimating Equation Analysis
Adjusted For Baseline HRQoL Values

HRQoL	NR vs DAS28 Remission		NR vs SDAI Remission		NR vs Boolean Remission	
	<i>Coefficient (95% CI)</i>	<i>P value</i>	<i>Coefficient (95% CI)</i>	<i>P value</i>	<i>Coefficient (95% CI)</i>	<i>P value</i>
<i>HAQ</i>	-0.27 (-0.56 , 0.02)	0.065	-0.41 (-0.81, -0.22)	0.038	-0.36 (-0.76, 0.03)	0.07
<i>Eq5D</i>	0.12 (0.07, 0.18)	<0.0001	0.16 (0.103, 0.22)	<0.0001	0.19 (0.13, 2.46)	<0.0001
<i>FACIT-F</i>	5.11 (1.13, 8.96)	0.009	9.97 (5.19, 14.76)	<0.0001	9.86 (4.82, 14.90)	<0.0001
<i>SF36 PCS</i>	5.03 (2.41, 7.65)	<0.0001	8.07 (5.6, 10.53)	<0.0001	8.39 (6.49, 10.28)	<0.0001
<i>SF36 MCS</i>	4.22 (1.56, 6.88)	0.002	5.86 (2.53, 9.18)	0.001	7.04 (2.96, 11.05)	0.001

7.4 Discussion

In the REMIRA cohort, significant differences were found in HRQoL outcomes within the different levels of low disease activity. The positive impact of point remission can be seen with all HRQoL at baseline and over time with all aspects of HrQoL outcomes when compared to LDAS. The impact of sustained remission on HRQoL is dependent on the remission criteria used. With the more lenient DAS28ESR and SDAI remission criteria, all 4 HRQoL outcomes showed improvement with increasing frequency of remission over time. With the most stringent ACR/EULAR Boolean remission criteria, the effects of intermittent and sustained remission were similar across all 4 HRQoL.

HAQ has been shown to be a predictor of remission (Eberhardt, Fex 1998, Gossec et al. 2004). This study has shown that HAQ was also significantly lower in all 3 point remission criteria compared to low disease activity state. This was also shown in the BARFOT study (Svensson et al. 2013). The minimum clinically important difference (MCID) has been reported as greater than 0.22 in clinical trials (van Riel et al. 2008), however, the change in clinical practice has been reported as lower at 0.09 (Pope et al. 2009). The difference between LDAS and remission between any of the criteria were larger than the MCID. In the literature, HAQ score <0.5 is considered as remission (Molenaar, Voskuyl & Dijkmans 2002). However, the REMIRA study has demonstrated that levels achieved were lower than this (upper quartile range 0.125-0.5). The median HAQ were all 0 in the least stringent DAS28ESR remission group and the stringent SDAI and Boolean groups suggesting that the assessment of HAQ in LDAS may have a floor effect. This may

also account for the results of the GEE analysis where there was no difference between LDAS and DAS28ESR after correcting for baseline HAQ. Disability and different remission criteria were explored in the DREAM cohort using anti-TNF. Their reported HAQ levels were higher than those in our study (de Punder et al. 2012). This is likely to be due to the fact that the DREAM cohort was more likely to have more severe disease given that they were all taking anti-TNF. The REMIRA median HAQ value was also lower than the original cut-off for predictive validity of the ACR/EULAR Boolean criteria (Felson et al. 2011).

The minimum clinically important change (MCID) of Euroqol is 0.05 and EuroQol VAS of >82 is representative of population norms have been used in a previous study of RA (van Riel et al. 2008). Although the EuroQol and EurQol VAS values were high throughout our whole cohort, there were clinically meaningful differences in EuroQol between LDA and remission in all 3 remission criteria groups. With the more stringent criteria, EurQol scores were at the maximum level. In addition, the median EuroQol VAS (80) in DAS28ESR remission was just under the population norm, whereas in both the more stringent criteria SDAI and Boolean, their median VAS in the remission groups were higher at 90. There was a clear impact of point remission on EuroQol and EuroQol VAS over time. There was also a clear difference in better EuroQol with increasing frequency of DAS28ESR and SDAI remission. The EuroQol levels between SDAI and LDAS at baseline were comparable that found in the literature (Radner, Smolen & Aletaha 2014).

SF36 have extensive evidence on reliability and validity. It has demonstrated responsiveness to change in patients with RA (Ruta et al. 1998). The advantage of using SF36 is that it is compared to norms from a population of subjects with no known history of specified illness. The levels found in the REMIRA study were comparable to the literature (Radner, Smolen & Aletaha 2014). Across all 3 point remission, the SF36 PCS is the same as the population norm whereas the SF36 MCS is higher than the population norm. It is interesting to note that SF36 MCS values are higher than PCS in both LDA and remission groups. The MCID in both SF36 MCS and PCS were 3 (Kosinski et al. 2000). This was reached in all 3 remission criteria compared to LDA groups. SF36 MCS and PCS improved. GH domain was the same between LDAS and DAS28IR as well as SDAI IR. Improvement was only seen in sustained remission. This again shows the importance of being in sustained remission.

Fatigue is a common symptom in the majority of patients with RA and has been recommended to be an outcome measured in RA studies (Kirwan et al. 2007). FACIT-F has been validated for use in RA and the MCID has been reported as 3.56 (Cella et al. 2005). The REMIRA cohort showed that patients in all 3 point remission states had clinically significant less fatigue than LDAS patients. This impact was seen throughout whole year of follow-up.

In all HRQoL measures, there was no difference between intermittent and sustained Boolean remission. This suggests that with stringent remission, intermittent remission maybe good enough to achieve maximal quality of life.

In conclusion, HRQoL outcomes are the most important outcomes from the patient perspective and should be given as much importance as other RA outcomes. Point remission allows patients to achieve near normal function with significant impact on their disease over time. When considering sustained remission, Boolean remission may be 'over-stringent' since no differences were found between intermittent and sustained remission. This implies that any patients achieving Boolean remission is likely to have a better outcome. This study has demonstrated that in routine clinical practice, even patients in low disease activity states have limited HRQoL and therefore LDAS should not be used as a treatment goal for RA.

8 Discussion

The findings of this thesis have made a number of novel contributions towards understanding of remission in RA. There are three important findings. Firstly, the definition of remission and sustained remission have been characterised in detail using an extended range of clinical, laboratory and radiological biomarkers. Secondly, predictors of remission, both for remission at single time points and also for remission sustained over periods of time have been identified; these could guide treatment decisions in clinical settings. Thirdly, the study has shown the importance of aiming for sustained remission over low disease activity states to maximize the benefits to health related quality of life.

8.1 Synopsis of key findings

The systematic review found that remission is becoming a realistic therapeutic target in early RA. Observational studies showed an overall remission rate of 17% with modified old ACR remission criteria and 33% with DAS remission criteria. The RCTs showed that more patients achieved remission when combination treatments were used (random OR 1.69–2.01 compared to DMARD monotherapies). Radiological progression was less in patients receiving combination therapies who were in remission. Despite this, patients in clinical remission still had ongoing radiological progression. This suggests that true remission remains ill-defined using the available remission criteria.

A model was then created to predict which patients are more likely to achieve remission after 24 months of treatment. The Remission Score using simple clinical assessments correctly classifies over 70% of patients. The score is equally applicable in the clinical trial setting (CARDERA) as well as in the routine practice setting (ERAN cohort). Three variables – gender, age and baseline tender joint counts – are all that is required. There was a marked difference in the likelihood of remission between different groups. Males who are under 50 years of age with less than 6 tender joints were the most likely to be in remission at 24 months. The Simplified Remission Score is readily applied in routine practice, and is similar in approach to the Simplified Disease Activity Index (Gaujoux-Viala et al. 2012). The prediction model had good specificity but poor sensitivity. This suggests that other biomarkers are likely to be required.

Next, it was assessed whether all patients responded to combination treatment in a similar fashion. Early intensive regimes have become the gold standard in the treatment of early RA. However, this study showed that the combination approach is only superior to monotherapy in certain subsets of patients. Stratifying patients according to gender, age, tender joint counts, RF IgM positivity and ACPA positivity can predict those subjects more likely to achieve remission states after 24 months of intensive treatment.

After establishing which patients are most likely to achieve in remission, the next part of the thesis was to explore what happened to patients once they achieved a stable

low disease activity states. To address this, patients in stable low disease activity states were recruited to the REMIRA cohort and followed up for 1 year. Baseline remission rates ranged from 30% (Boolean remission) to 66% (DAS28 ESR remission). 47%, 33%, 22%, 23% and 10% of patients achieved sustained DAS28ESR, DAS28 CRP, SDAI, CDAI and Boolean remission respectively. 10% of patients did not achieve remission by any criteria at any point during the follow-up period (No remission group). In total, 13.5% of patients developed erosive progression over the follow-up period. There were no differences in power Doppler (PD) signal and synovial hypertrophy (SH) at baseline between NR and any of the remission groups. NR patients had significantly more SH at T12 compared to all 3 remission groups. There was a similar trend for higher PD scores at T12 but this did not reach significance (p value range 0.051 – 0.074). Both SH and PD at baseline were predictors of radiographic progression in low disease activity states.

True remission should include stability of disease over time and therefore sustained remission should be the ultimate aim in therapy. Baseline predictors of sustained DAS28 and SDAI remission included ethnicity, ESR, CRP, TJC, Patient global assessment and physician assessment. Baseline predictors of sustained Boolean remission also included ESR, TJC, Patient global assessment, physician assessment but also the presence of erosions at baseline.

Reliable assessment of remission is important for the optimal management of patients with RA. Clinical assessments of disease activity have been criticised for being too subjective. Therefore more objective laboratory markers are required to supplement

clinical assessment of remission status. Analysis of serum samples from subjects enrolled in the REMIRA study has identified novel biomarkers which may contribute to defining low disease activity states. This cohort of patients who are clinically similar in phenotype shows marked heterogeneity at the molecular level. Despite this, we found several biomarkers which were able to define the different remission criteria. MBDA and some of its analytes (hsCRP, SAA, IL6, Leptin), calprotectin and CXCL10 were able to differentiate between LDAS and all 3 point remission criteria. In addition, TNFR1 and VCAM1 were able to differentiate between LDAS and DAS28ESR point remission. MBDA, hsCRP, SAA, IL6, leptin, CXCL10 levels at baseline or over 6 months were able to predict the frequency of remission over 12 months. Calprotectin levels over 6 months but not at baseline were able to predict the frequency of remission over 12 months.

Lastly, this thesis determined the impact of sustained remission versus low disease activity states from a patient's perspective. The impact of point remission can be seen with all HRQoL at baseline and over time with all aspects of HrQoL outcomes. The impact of sustained remission on HRQoL is dependent on the remission criteria used. With the more lenient DAS28ESR and SDAI remission criteria, all 4 HRQoL outcomes showed improvement with increasing frequency of remission over time. With the most stringent ACR/EULAR Boolean remission criteria, the effects of intermittent and sustained remission were similar across all 4 HRQoL.

8.2 Strengths and Limitations

8.2.1 Systematic review

Strengths

This was a comprehensive systematic review using observational studies and RCTs to assess the frequency of remission and the impact of treatment. An update was also carried out to ensure that the newer studies did not change the results of the systematic review.

Limitations

This systematic review has several limitations. One issue is study heterogeneity. The studies varied in duration (12-120 months), design (observational and trials), treatment approaches (DMARD monotherapy and intensive combination regimens) and the classification of remission (ACR and DAS criteria). Most studies used single time-points to define remission; this was usually at the end of follow-up. Those studies reporting remission rates over prolonged periods recorded fewer remissions. Another limitation is focusing on early RA, thereby excluding studies of patients with undifferentiated early inflammatory arthritis. The Norfolk Arthritis Register (NOAR) exemplifies such studies; it shows there are more remissions in milder forms of arthritis (Symmons, Silman 2006). Older “classic” studies going back several decades were excluded. Changes in the management of RA over the last 20 years mean these historical studies have limited current relevance. In addition, the difference between the effects of monotherapies versus combination DMARD therapies may be exaggerated due to the choice of DMARD in the monotherapy arm. SSZ is often used as

DMARD monotherapy and is considered by some experts to be a 'weaker' DMARD in comparison to MTX, though the relative efficacy of different DMARDs is a contentious issue. There is also controversy over whether patients treated with steroids, particularly at high dosages can be considered as being in remission. Some of the RCTs did use high dose steroids at the beginning of treatment but these were rapidly reduced to 7.5mg. Low dose prednisolone was considered as acceptable and have included these in the analysis. In addition, it is important to bear in mind that differences between groups of patients are easier to demonstrate when there are high potentials for progression as opposed to low potentials for progression; the same is true in showing differences between highly effective and relatively ineffective treatments. Lastly, the use of Jadad score may be over-simplistic. It has been criticised for not taking into account of allocation concealment.

8.2.2 Predictors of Remission at 24 months and treatment response

Strengths

The strength of the prediction model was that it was created using a large RCT dataset and then validated in a cohort study. Unfortunately, since there was no serum available in the ERAN cohort, the second part of the study assessing treatment response could not be validated with the same method. However, there is confirmatory evidence in another publication which used the same dataset. This showed again that only ACPA status influences the need for combination DMARDs (Seegobin et al. 2014).

Limitations

This study has several limitations. Firstly, it used data collected for other purposes and neither the clinical trial nor the observational study were powered to investigate remission. A far larger dataset is needed to investigate all the potentially relevant factors. Secondly, the treatments used in the RCT - Methotrexate, Ciclosporin and short-term high dose prednisolone - are not widely used as initial combinations in contemporary RA treatment. The findings in this study might not be generalisable to all intensive combination therapies. However, it is a well-recognised combination and many RCTs have demonstrated its efficacy. Ciclosporin is infrequently used in RA, though there is extensive evidence base for its use, which has been summarised in a Cochrane review by Wells et al. (Wells et al. 2000). Although it is both effective and relatively safe, other DMARDs like Sulfasalazine and Hydroxychloroquine are usually given in combination with Methotrexate. Thirdly, the RCT used fixed treatment regimens rather than the treat-to-target approach which is now widely used in early RA management. Further research is needed to assess the benefits and risks of “treat-to-target strategies in ACPA negative disease. Fourthly, DAS28 remission criteria was used because it is readily achievable in clinical practice. Stricter remission criteria may be preferable in the longer term, such as the ACR/EULAR Boolean remission criteria. Fourthly, using a single time point at 2 years as the sole criterion for judging remission is insufficiently rigorous and ideally extended periods of remission would be a more clinically relevant target. However, a far larger database would be required for this purpose. Finally, although the specificity of our remission score is high, its sensitivity is relatively poor. The use of a more extended range of clinical and

laboratory markers might improve the prediction of remission in future and more research is needed in this area.

8.2.3 The REMIRA cohort

Strengths

The strength of the REMIRA cohort is firstly that this is a large and unique cohort of RA patients with LDAS with detailed clinical assessments and a large biobank of biological materials collected serially over time. The second strength of this cohort lies in the frequency of follow-ups. Most papers reporting sustained remission over years with yearly follow-ups. As this study has demonstrated, in this unique cohort of patients with stable LDAS, disease activity can still be dynamic over a year. Therefore, the definition of sustained remission in this study which included being in remission at every time point 3 months apart for 1 year was very stringent. The original ACR remission criteria in the seminal report by Pinals et al in 1981 concluded 'complete' RA remission indicates the 'total absence of articular and extraarticular inflammation and immunological activities'. Despite advances in therapies, this criteria is still too difficult to achieve. Within their criteria, they also incorporated time into the definition of remission. They chose 2 consecutive months. Other reports of sustained remission opted for much longer periods of remission. Jayakumar et al, 2012 (Jayakumar et al. 2012) looked at sustained remission over 5 years and Prince et al., 7 years (Prince et al. 2012). Currently, there is no consensus on what the optimal time should be. Within the REMIRA study, 1 year was chosen as it was thought to be a good balance between assessing the stability of disease activity and feasibility in clinical

practice. The agreement between the sustained remission criteria was slightly better than point remission. Thirdly, the strength of the ultrasound aspect of the study is that the same ultrasound machine was used for all patients with the same operator. There was good inter- and intra-observer agreement between the first and second readers.

Limitations

The main limitation of the study was related to the small number of patients in sustained remission and in the comparator group (NR group). Even though this remission cohort is more stringent than other cohorts previously reported (Brown et al. 2008), given the low prevalence of Boolean sustained remission and NR group, analysis was limited. In particular, multivariate logistic regression was not possible. Secondly, radiographic progression was defined as a binary outcome (new or worsening erosions). This may not have been a sensitive enough outcome for a cohort with similar disease activity. Other scoring methods may have been more suitable. However, it was decided that a binary outcome was preferable as there was less potential noise. Thirdly, as the patients in the different remission criteria overlapped with each other, it was not possible to compare parameters between the groups. Fourthly, BMI was not collected for the study and therefore it was not possible to correct for BMI when analyzing leptin levels.

8.3 Clinical implications of study

The findings of each chapter have been discussed in detail within the individual sections. This section of the general discussion is to highlight the clinical implications of these findings.

8.3.1 Personalised medicine

In the era where personalised medicine is becoming a reality, treatment of RA patients should be more individualised. This thesis suggests that both the remission score and serological status could be used as therapy decision tools. The results suggest that initial combination therapy may only be useful in certain subsets of early RA patients. It is premature to use the remission score for treatment decisions in routine practice; further work is required to validate this approach and to improve its specificity. Other biomarkers are likely to be required to achieve a personalised approach for the treatment of RA. However, the concept of a predictive remission score would be useful to tailor treatment regimes at an individual patient level. The findings of the study challenge the established view that all RA patients should be given intensive combination treatment as recommended by NICE guidance. This study favours the more cautious approach in the 2013 EULAR guidance.

8.3.2 Clinical and laboratory remission biomarkers

The thesis has shown several clinical and laboratory remission biomarkers. Table 8.1 summarised the continuous variables in order of effect size as defined standardized ORs. Baseline SAA was the best laboratory predictor of sustained DAS28 and SDAI remission whereas physician global assessment scores were the best clinical

predictors. It is interesting that similar predictors were found in both sustained DAS28 and SDAI remission. Baseline ESR, TJC and PhGA predicted Boolean remission perfectly. MBDA score was a predictor in all 3 sustained remission criteria but only a few of these biomarkers within the panel were shown to be important in predicting sustained remission. Out of the binary variables, ethnicity was a good predictor of sustained DAS28 and SDAI remission (ORs= 0.06 and 0.10 respectively) and having erosions on x-rays at baseline was a good predictor of boolean remission (OR = 13.50)

The methodology used to create the remission score in Chapter 4 can be applied to sustained remission. Once the above variables can be validated as predictors of sustained remission, a clinical and extended REMIRA score can be developed, similar to the idea of SDAI and CDAI scores. These will be very useful in clinical practice. These scores will be able to inform when patients are likely to remain in stable true remission over 1 year. If this can be determined, drug tapering can be considered.

Table 8-1: Summary of the predictors of sustained remission.
The table summarises the continuous clinical and laboratory variables in order of standardized Odds ratios. * AUC value is used over baseline value as this preformed much better as a predictor.

Remission Type	Baseline Variable	Standardised OR
<i>DAS28</i>	Serum Amyloid A	0.01
	Erythrocyte Sedimentation Rate	0.02
	C-Reactive Protein	0.05
	Physician Global	0.09
	Patient Global	0.15
	Calprotectin AUC*	0.19
	Tender Joint Count	0.25
	CXC motif chemokine 10	0.31
	Multi-biomarker Disease Activity	0.36
	High sensitivity C-Reactive Protein	0.38
<i>SDAI</i>	Leptin	0.54
	Physician Global	Predicts perfectly
	Patient Global	0.02
	Serum Amyloid A	0.03
	Tender Joint Count	0.07
	C-Reactive Protein	0.09
	Calprotectin AUC*	0.11
	Erythrocyte Sedimentation Rate	0.25
	Multi-biomarker Disease Activity	0.28
	High sensitivity C-Reactive Protein	0.36
<i>Boolean</i>	Leptin	0.45
	Erythrocyte Sedimentation Rate	Predicts perfectly
	Tender Joint Count	Predicts perfectly
	Physician Global	Predicts perfectly
	High sensitivity C-Reactive Protein	0.03
	AUC*	
	Patient Global	0.04
	Multi-biomarker Disease Activity	0.34

8.3.3 Role of ultrasound in remission

From the systematic review, it is clear that sub-clinical inflammation is present in a significant proportion of RA patients in clinical remission leading to radiographic progression. This was confirmed in the REMIRA study. It is established that the presence of a PD signal predicts radiographic progression in low disease activity states (Brown et al. 2008, 618, Foltz et al. 2012), however, the role of SH is less clear. Boyesen found SH predicted 1 year MRI erosive progression in patients with active disease (Boyesen et al. 2011). The REMIRA study has demonstrated for the first time that synovial hypertrophy, as well as power Doppler, has a role in predicting radiographic progression in low disease activity states. In addition, synovial hypertrophy was also found to be less in all sustained remission groups at the end of the study compared to the LDAS group. This suggests that synovial hypertrophy can be reduced with stringent sustained remission. The clinical implication of this is treatment escalation should be considered in patients with LDAS who have power Doppler signal and/or synovial hypertrophy.

More stringent criteria have been shown to be closely associated with less sub-clinical synovitis. Balsa et al., 2010, showed the superiority of SDAI over DAS28 remission criteria in the assessment of ultrasound-classified remission (Balsa et al. 2010). However, this was not substantiated in a paper by Saleem et al., 2011 which showed similar PD signal between DAS28, SDAI and Boolean remission (Saleem et al. 2011). The REMIRA study also did not find a role for ultrasound as a predictor of sustained remission.

8.3.4 Aim for remission, not LDAS, to optimise HrQOL

Chapter 7 established that in clinical practice, in order to optimise quality of life of patients with rheumatoid arthritis, the aim of treatment should be to achieve sustained DAS28, sustained SDAI or at least intermittent Boolean remission. The REMIRA study has shown that disease activity can fluctuate over 1 year. In order to achieve sustained remission, it may be necessary to keep patients under closer follow-up than may be done in routine practice currently, where patients with LDAS or remission states are routinely followed up on a yearly basis.

8.4 Future studies

8.4.1 The use of predictors of sustained remission in drug-tapering

With the growing number of RA patients reaching remission, the new clinical problem is knowing how best to treat these patients once they achieve sustained remission. Once remission can be identified with certainty, tapering of DMARD treatment after sustained remission should be the next goal in treatment. Several papers have been published on drug tapering and withdrawal. The BeST study which compared 4 different treatment regimes in early RA patients: DMARD monotherapy, step up DMARD combinations, step-down DMARD combinations and infliximab with Methotrexate. When patients achieved remission, DMARDs were tapered and stopped. 23% of patients achieved drug-free remission during the 5 year follow-up period (Klarenbeek et al. 2011). A meta-analysis of DMARDs withdrawal or tapering showed that the relative risk of flares in patients remaining on DMARDs compared to patients in whom DMARDs were stopped was 0.31 (95% CI 0.16, 0.57) (O'Mahony et al. 2010). This suggests that further work is required to establish reliable predictors of sustained remission upon drug-tapering.

Currently, a follow-up study to the REMIRA is ongoing which will investigate the effects of biologics drug-tapering in patients with LDAS. This study is named: Optimising Treatment With Tumour Necrosis Factor Inhibitors In Rheumatoid Arthritis: Is Dose Tapering Practical In Good Responders? (OPTTIRA study, Table 8.2). This randomised trial will study the following in patients tapering TNF inhibitors and controls:

- a. The risk of disease flares (DAS28 increases ≥ 0.6).
- b. If flares are reversed by reverting to the original TNF inhibitor dosage.
- c. If either tapering group shows worse key RA assessments including disease activity (DAS28) and disability as measured by health assessment questionnaire (HAQ) scores.

Ultimately, an RCT should be designed to address DMARDs drug-tapering directly. Once predictors of sustained remission are established, a remission algorithm could be established to guide treatment taper. To test the validity of this approach, an RCT with 3 treatment arms will be required:

- 1) Standard Care Treatment with no tapering of medication
- 2) Standard Care treatment with tapering of DMARDs which will be guided by clinicians
- 2) DMARD drug tapering based on the remission algorithm.

Table 8-2 Protocol for the OPTTIRA Study

TITLE
Optimising Treatment With Tumour Necrosis Factor Inhibitors In Rheumatoid Arthritis: Is Dose Tapering Practical In Good Responders? A “Proof Of Principle” And Exploratory Trial.
HYPOTHESIS
Tapering TNF inhibitors (to a minimum of one third of the initial “induction” doses) will not adversely affect disease control in established RA patients who have achieved a good response to standard doses of TNF inhibitors and are also receiving disease modifying anti-rheumatic drugs (DMARDs). We consider an increase of disease activity score (DAS28) ≥ 0.6 represents a clinical important change.
TRIAL DESIGN
Randomised controlled, open label multicentred, proof of principle trial followed by open exploratory phase trial in patients with established RA who have achieved a good response to standard doses of TNF inhibitors and are also receiving disease modifying anti-rheumatic drugs (DMARDs). Follow-up period = 12 months
INCLUSION CRITERIA
<ul style="list-style-type: none"> • RA by American College of Rheumatology and EULAR criteria • Etanercept or Adalimumab treatment for at least 6 months (a break of up to 4 consecutive weeks is permitted). • Taking at least one DMARD • Stable clinical response for ≥ 3 months (one DAS28 score ≤ 3.2; no increase in DAS28 > 0.6) • Patient considers he or she has achieved a suitable response to TNF inhibitors. • Supervising rheumatologist considers further improvements are unlikely on the patient’s current treatment regimen. • At least 18 years of age. • Willing and able to give informed consent.
EXCLUSION CRITERIA
<ul style="list-style-type: none"> • Serious concurrent illness (e.g. terminal cancer). • Prednisolone at more than 10mg daily (for doses > 10mg daily, a 4 week washout period is required). • Recently received IM/IA steroids (12 weeks washout required) • Pregnancy, breast-feeding or women of child-bearing potential not using adequate contraception
SAMPLE SIZE
99 Patients.
ASSESSMENTS
Visits at baseline, 3, 6, 9 and 12 months using standard validated questionnaires, and there will be monthly telephone calls between visits. X-rays of hands and feet will be taken at baseline, 6 months and 12 months. Patients will be monitored via blood tests as set out in current guidelines for all DMARDs and TNF inhibitors.
BIOMARKER SUB-STUDY
The study will also include an optional Biomarker Sub-study which will aim to identify patients who are in true remission, based on clinical, imaging and laboratory parameters. These parameters will be used to predict those subjects most likely to tolerate TNF tapering and/or withdrawal in the OPTTIRA study. Study subjects in true remission would be predicted to best tolerate drug withdrawal.
The Sub-study is optional for participants and blood samples will be collected from experimental groups 1 and 2 at the baseline assessment and at the 6 month assessment for participants in the control group.

8.4.2 Ongoing Research

The REMIRA study has contributed to the development of 2 new studies:

a) Towards a cure for early Rheumatoid arthritis (TACERA study, Table 8.3). This is longitudinal observational cohort study with early seropostive RA patients. The aims of this study are firstly to apply a combination of clinical and laboratory parameters to predict clinical responses to disease modifying drugs in patients with recent onset RA; secondly to use laboratory parameters to monitor biological responses to therapy; and thirdly define a true biological remission state in patients with early RA. Such an approach to therapeutic decision-making means that patients receive the drug combinations most likely to induce and sustain remission. The clinical and laboratory biomarkers from the REMIRA study have helped with the development of these 3 aims.

b) Treatment intensities and targets in rheumatoid arthritis therapy (TITRATE) study (Table 8.4). This is a RCT comparing the intensive management compared to standard care on remission rates at 12 months in rheumatoid arthritis patients with intermediate disease activity. The primary endpoint of the study is to assess the number of patients in each treatment arm fulfilling the definition of remission as measured by DAS28-ESR. The role of the novel biomarkers identified in the REMIRA study will also be investigated in this study.

Table 8-3 Protocol for the TACERA Study

Study Design
A longitudinal observational study of an early RA inception cohort over 18 months
Working Hypothesis
A suite of immunological assays (“the immunological toolkit”) can be used to accurately predict clinical responses to therapy at a molecular and cellular level. We also propose that immune based assays can be adapted to define an immune signature associated with a state of sustained clinical remission in patients with early RA. This study protocol seeks to recruit a large cohort of patients with early RA. Biological samples will be acquired from study subjects and used to develop the immunological toolkit, through the identification of baseline biomarker signatures (prior to starting therapy), and by documenting the changes in the immune system in response to therapeutic intervention.
Key Inclusion Criteria
<ul style="list-style-type: none"> • Diagnosis of early RA using either 1987 ACR or the 2010 ACR/EULAR criteria • Positive for serum rheumatoid factor and anti-citrullinated protein autoantibodies (ACPA) • Within 12 months of symptom onset • Able and willing to give informed consent to provide clinical data and blood samples at defined time points for the duration of the study • Aged over 18 years
Key Exclusion Criteria
<ul style="list-style-type: none"> • Previous treatment with DMARDS or biologics • Corticosteroid treatment for the current episode of inflammatory arthritis within the last 12 months • Pregnant • Significant co-morbidities
Study treatment
The study is observational an observational study where patients will receive routine clinical care according to NICE guidelines.
Sample Size
410 Patients.
Assessments:
Visits will comprise a detailed assessment of disease activity (including standard blood monitoring for DMARDS, TNF inhibitors or other biological agents, as appropriate) along with completion of a number of questionnaires and the provision of additional blood and urine samples for immunoanalysis. X-rays of the hands and feet will be taken at Baseline, 12 and 18 months. Where Ultrasound scanning is offered as part of routine care participants may have high resolution ultrasound scanning (HRUS) of affected joints performed at Baseline, 6, 12 and 18 (optional) months.

Table 8-4 Protocol for the TITRATE Study

Study Design
A pragmatic randomised controlled open trial of the effect of intensive management (IM) compared with standard care (SC) on remission rates at 12 months in rheumatoid arthritis patients with intermediate disease activity.
Working Hypothesis
Patients with established RA (of at least 6 months duration), who currently have intermediate disease activity (defined as DAS28-ESR 3.2-5.1 with at least 3 active joints) and are currently receiving at least one DMARD, are more likely to achieve remission at 12 months if they receive intensive management than if they continue to have standard care.
Key Inclusion Criteria
<ul style="list-style-type: none"> • Aged over 18 years • Diagnosis of rheumatoid arthritis for 6 months-10 years • Have intermediate disease activity (DAS28-ESR 3.2-5.1 with at least 3 active joints) • Have received at least one DMARD for a period of six months or more.
Key Exclusion Criteria
<ul style="list-style-type: none"> • Participants who have failed ≥ 5 DMARDS or have received biologics • Have irreversible disability from extensive joint damage • Have major comorbidities (e.g., heart failure) • Are pregnant, breast-feeding or at risk of conceiving • Are unable or unwilling to give informed consent • Are currently or have recently participated in another interventional trial or are currently in an early RA pathway.
Study treatment
<p>Intervention group: Intensive Management will involve monthly clinical reviews which will allow immediate adjustments of patients' management in response to their clinical status. Part of the management will be the optimal intensive use of standard drug treatments. These will all be given within their licensed indication, at licensed doses and routes of administration. Intensive management will also involve supportive non-drug interventions which will be individualised to meet patients' specific needs. These non-drug approaches will be combined in a 'treatment support' programme. They will span psychoeducation, goal-setting and skills teaching to address identified problem areas, such as pain, fatigue and low physical activity, and will be outlined in a treatment support manual.</p> <p>Comparator group: Standard care, which consists of at least one routine clinical review.</p>
Sample Size
398 Patients.
Assessments:
All patients will be assessed at baseline, 6 months and 12 months. In addition, the intensive group will be assessed monthly for treatment changes according to protocol in the first 6 months.

8.4.3 Peripheral blood mononuclear cells signatures of remission (PBMCs)

Adaptive immunity underpins RA synovitis, shown by its strong HLA-DRB1 association, confirmed in genome wide association studies, and the efficacy of costimulatory blockade and B cell therapies (Cope 2008, Kremer et al. 2003, Edwards et al. 2004). But the use of cell based immunoassays for clinical decision making is less advanced. There is a need to identify immune cell signatures reflecting disease activity; identifying LDA signatures is one possible example. Four subsets of atypical CD4⁺ T lymphocytes are implicated in RA pathogenesis and persistence. Unlike conventional Th effector T cells, whose differentiation depends on antigen specific TCR ligation, unconventional subsets emerge in response to chronic inflammatory cytokine signals:

- Subset Of Inflammation Related Cells (IRC): these reflect a distinct CD45RB^{bright}, CD45RA⁺, CD45RO^{dim} and CD62L⁻ phenotype; cytokine drive may be important for expansion and/or maintenance of IRC (Ponchel et al. 2002).
- Cytokine stimulated T cells (Tck): this cell population, identified by Brennan and colleagues, resemble RA synovial T cells; they are potent inducers of contact-dependent TNF production by monocytes (Brennan et al. 2002, Brennan et al. 2008).
- Antigen Experienced T Cells with reduced expression of TCR α (TCR α ^{dim}): several groups, including our own, have identified these cells, which migrate to inflammatory sites like RA synovia, and accumulate in peripheral blood (PB) when migration is blocked in vivo with TNF blockers (Zhang et al. 2007, Maurice et al. 1997).

Atypical, cytokine driven T cells share the expression of cell surface antigens associated with abnormal or terminal differentiation, persistence of effector function and enhanced migratory competence. Exclusion of IRC and TCR α dim T cells from peripheral tissues and their accumulation in PB is linked to remission (Burgoyne et al. 2008). Interestingly, persistence of IRC in RA patients achieving remission predicted disease flares in >70% patients within 18 months. Their persistence implies incomplete suppression of synovitis, undetectable using conventional clinical assessments. These results suggest that:

- 1) Effector T cell subsets: these accumulate in PB in response to DMARD or biological therapy, as a consequence of endothelial cell de-activation and inhibition of cell migration.
- 2) Increased atypical T cells: will identify patients with erosive disease likely to flare on tapering DMARDs
- 3) Low numbers of unconventional T cells in PB: should predict stable LDA without erosive progression.

Distinguishing these subsets of patients could help define patients in 'immunological' remission. During the REMIRA study, PBMCs were collected at all timepoints and stored in liquid nitrogen. It is likely that immunophenotyping using flow-cytometry will also contribute to a more accurate definition of true remission and hence in turn, play a role in predicting sustained remission

8.4.4 Gene expression profiling to predict flares

This technology has evolved from studying selected gene sets to whole genome screening where all genes expressed are sampled at single time points. Microarray technology permits the acquisition of comprehensive profiles (gene expression signatures) unique to a tissue or cell type. This unbiased approach also: (i) identifies the coordinated expression of large panels of known genes; (ii) provides insights into common pathways of gene regulation; (iii) may identify unique patterns of novel genes; (iv) facilitates the identification of novel therapeutic targets in distinct patient subsets. No published studies report comprehensive analysis of remission-specific gene expression signatures in RA, with the exception of a recent study of spontaneous (drug free) remission in a cohort of pregnant RA patients (Haupt et al. 2008). Pregnant RA patients had similar PB gene expression profiles to healthy women in the third trimester, showing that in drug free remission biological signatures normalise in RA patients. Identifying related signatures in RA patients with LDA states would be a key advance as it could identify patients suitable for treatment tapering or withdrawal.

The REMIRA cohort will be used to explore the role of gene expression in predicting flares. Baseline samples of patients who flared during follow-up were compared to patients who have remained in sustained remission. These samples were matched for age, gender, treatment and disease activity at baseline. Transcriptomic data on over 70 samples have been acquired so far. Analysis is ongoing currently.

8.4.5 Health economics of sustained remission.

Whilst the impact of sustained remission on the quality of life was evaluated in this study, its cost-effectiveness has not been explored. A systematic review showed that treatment strategies leading to maintenance of physical function and keeping patients at work are cost effective even when including biological agents (Schoels et al. 2010). A recent study by Radner et al showed that patients in point remission had better work productivity, lower indirect and direct costs when compared to LDAS state (Radner, Smolen & Aletaha 2014). HAQ and EuroQol have been used to calculate cost-effectiveness of biological therapies in NICE guidance (NICE 2009). Therefore, a similar approach can be taken to assess the cost-effectiveness of keeping patients in sustained remission.

9 Conclusions

Remission is the ultimate goal when treating patients with RA. The seminal report by Pinals et al in 1981 suggested that “complete” RA remission should indicate the “total absence of articular and extraarticular inflammation and immunological activities”. Since that time, there have been uncertainties on how best to define remission states. This thesis had made contributions to knowledge about how to define remission and also have important implications for clinical practice.

The impact of the length of time in remission has been demonstrated. True remission implies stability over time. The treat-to-target approach is the gold-standard treatment for RA. Guidelines propose close monitoring of patients are required for intensive escalation of treatment for patients with active disease. This thesis study shows that even after reaching remission, strict monitoring is required, as only a small proportion of patients achieve sustained remission.

Targeted treatment should take into account the relative chance of entering state of sustained remission. Not all patients have the same likelihood of achieving remission. In an era where treatments are increasingly targeted at a molecular level, it is important that monitoring treatment responses progresses to reflect such specific therapies. Laboratory biomarkers, beyond ESR and CRP, are likely to play crucial roles in defining disease activity and remission in the future. This thesis has identified several novel serum biomarkers that could differentiate small changes in disease activity even at the low end of the disease activity. Molecular markers of inflammation

may be included in the evaluation of patients with low disease activity states to define remission states. The use of clinical predictors and as well as these novel biomarkers may help to stratify patients who are more likely to stay in sustained remission and help in decision making with treatment changes.

With the growing emphasis on personalised medicine, this thesis brings us one step closer to achieving individualised care. Ultimately, these findings can be used to define true remission and allow treatment to be tapered. This in turn will reduce the cost and toxicity of treatment, benefiting the NHS and patients alike.

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11 Appendix

11.1 Publications

Original Papers

1. Clinical and serological predictors of remission in Rheumatoid Arthritis is dependent on treatment regimes. Ma MHY, Scott IC, C Dahanayake, Cope AP and Scott DL. Accepted for publication *J Rheum*
2. ACPA-positive and ACPA-negative rheumatoid arthritis differ in their requirements for combination DMARDs and corticosteroids: secondary analysis of a randomized controlled trial. Seegobin SD, Ma MH, Dahanayake C, Cope AP, Scott DL, Lewis CM, Scott IC *Arthritis Res Ther*. 2014 Jan 16;16
3. Randomised controlled trial of tumour-necrosis-factor inhibitors against combination intensive disease modifying anti-rheumatic drugs in established rheumatoid arthritis: The TACIT trial DL Scott, F Ibrahim, V Farewell, AG O'Keeffe, MHY Ma, D Walker, M Heslin, A Patel, G Kingsley. Accepted for publication to HTA
4. Intracellular complement activation sustains T cell homeostasis and mediates effector differentiation. MK Liszewski, M Kolev, G LeFrieck, M Leung, PG. Bertram, A Fara, M S, MC.Pickering, C Drouet, S Meri, T. Petteri Arstila, PT. Pekkarinen, MHY Ma, AP Cope, T Reinheckel, S Rodriguez de Cordoba, B Afzali, JP Atkinson, C Kemper *Immunity*. 2013 Dec 12;39(6):1143-57
5. Remission in Early Rheumatoid Arthritis: Predicting Treatment Response. Ma MHY, Ibrahim F, Walker D, Hassell A, Choy EH, Kiely PD, Williams R, Walsh DA, Young A, Scott DL. *J Rheum* 39 (3):470-5. Also published in Clinical highlights for Rheumatologists March 2012 Volume 1, Issue 3 Page 1
6. Safety of combination therapies in early RA: a systematic comparison between combination DMARDs and TNF inhibitors with Methotrexate. Ma MHY, Cope AC, Scott DL. *Int. J. Clin. Rheumatol*. 2010 5(5), 547–554
7. Remission in Early Rheumatoid Arthritis. Ma MHY, Scott IC, Kingsley GH, Scott DL. *J Rheum* 2010 37:1444-53

Reviews

1. Editorial: Adding New Perspectives To The Kaleidoscope Of Remission Criteria in Rheumatoid Arthritis. MHY Ma, AP Cope, DL Scott *J Rheum* 40(4):353-5

11.2 Presentations at conferences

- 2014 BSR conference, Liverpool, Poster presentation
The REMIRA study: The impact of sustained remission on health-related quality of life in rheumatoid arthritis patients with low disease activity state.
- 2013 EULAR conference, Madrid. Poster: A multi-biomarker disease activity (VECTRADA algorithm) score and components are associated with sustained clinical remission in rheumatoid arthritis: The REMIRA study.
- 2013 BSR conference, Birmingham. Poster presentations:
1. Rheumatoid Factor IgA (RF IgA) and Anti-cyclic citrullinated peptides antibodies (ACPA) – predictors of radiographic progression.
 2. Systematic review comparing combination DMARD therapy with anti-TNF plus Methotrexate in drug resistant Rheumatoid Arthritis
 3. Serological status - a predictor of response to intensive therapy in Rheumatoid Arthritis (Guided poster)
- 2013 Spring Meeting for Clinician Scientists in Training (Guided poster): Serological status - a predictor of response to intensive therapy in Rheumatoid Arthritis
- 2013 Best Practice in RA management, London (talk): What happens to patients in remission?
- 2012 BSR (Glasgow, Guided poster): Biomarker signatures in RA patients with LDA: The REMIRA study
- 2011 ACR conference (Chicago, USA, Poster): Biomarker signatures in RA patients with LDA: The REMIRA study
- 2011 BSR conference (Brighton, UK, Poster): Persisting Remission is essential to achieve low HAQ score in RA.
- 2010 ACR conference (Atlanta, USA, Poster) Safety of combination therapies in early RA: a systematic comparison between combination DMARDs and TNF inhibitors with Methotrexate.
- 2010 BSR Conference (Birmingham, UK): Talk: Treatment decisions in RA: Are we undertreating the elderly?

11.3 Jadad score

Items	Score
Was the study described as randomized?	0/1
Was the method used to generate the sequence of randomization described and was it appropriate?	0/1
Was the study described as double-blind?	0/1
Was the method of double-blinding described and was it appropriate?	0/1
Was there a description of withdrawals and dropouts	0/1
Deduct 1 point if the method used to generate sequence of randomised was described but inappropriate	0/-1
Deduct 1 point if the study was described as double-blinded but the method of blinding was inappropriate.	0/-1

11.4 MBDA algorithm.

Formulae used to estimate tender joint count, swollen joint count, and patient global assessment.

PTJC	$-26.72 + 3.243 * [\text{YKL-40}]^{1/10} - 11.97 * [\text{EGF}]^{1/10} + 15.72 * [\text{IL-6}]^{1/10} + 0.4594 * [\text{Leptin}]^{1/10} + 3.881 * [\text{SAA}]^{1/10} + 0.7388 * [\text{TNF-RI}]^{1/10} - 0.2557 * [\text{VCAM-1}]^{1/10} + 0.7003 * [\text{VEGF-A}]^{1/10}$
PSJC	$-26.63 + 3.232 * [\text{YKL-40}]^{1/10} - 11.93 * [\text{EGF}]^{1/10} + 15.67 * [\text{IL-6}]^{1/10} + 0.4578 * [\text{Leptin}]^{1/10} + 3.868 * [\text{SAA}]^{1/10} + 0.7363 * [\text{TNF-RI}]^{1/10} - 0.2548 * [\text{VCAM-1}]^{1/10} + 0.6979 * [\text{VEGF-A}]^{1/10}$
PPG score	$-13.489 + 5.474 * [\text{IL-6}]^{1/10} + 0.486 * [\text{SAA}]^{1/10} + 2.246 * [\text{MMP-1}]^{1/10} + 1.684 * [\text{Leptin}]^{1/10} + 4.14 * [\text{TNF-RI}]^{1/10} + 2.292 * [\text{VEGF-A}]^{1/10} - 1.898 * [\text{EGF}]^{1/10} + 0.028 * [\text{MMP-3}]^{1/10} - 2.892 * [\text{VCAM-1}]^{1/10} - 0.506 * [\text{Resistin}]^{1/10}$

All biomarker concentrations are in pg/ml.

EGF = epidermal growth factor; IL-6 = interleukin-6; MMP = matrix metalloproteinase; PPG = predicted patient global; PSJC = predicted swollen joint count; PTJC = predicted tender joint count; SAA = serum amyloid A; TNF-R1 = tumor necrosis factor receptor superfamily member 1A ; VCAM-1 = vascular cell adhesion molecule 1; VEGF-A = vascular endothelial growth factor-A; YKL-40 = human cartilage glycoprotein 39.

11.5 Example of the follow-up page on the REMIRA ACCESS database

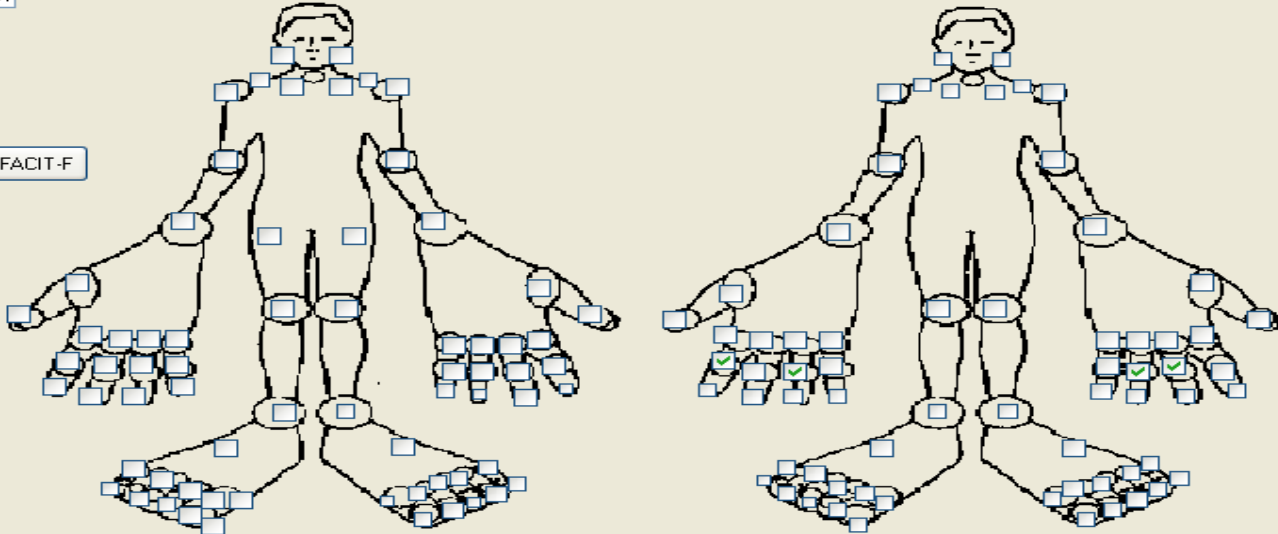
Patient Assessments Current and Past Medication

Hospital number: Date of assessment: Visit:

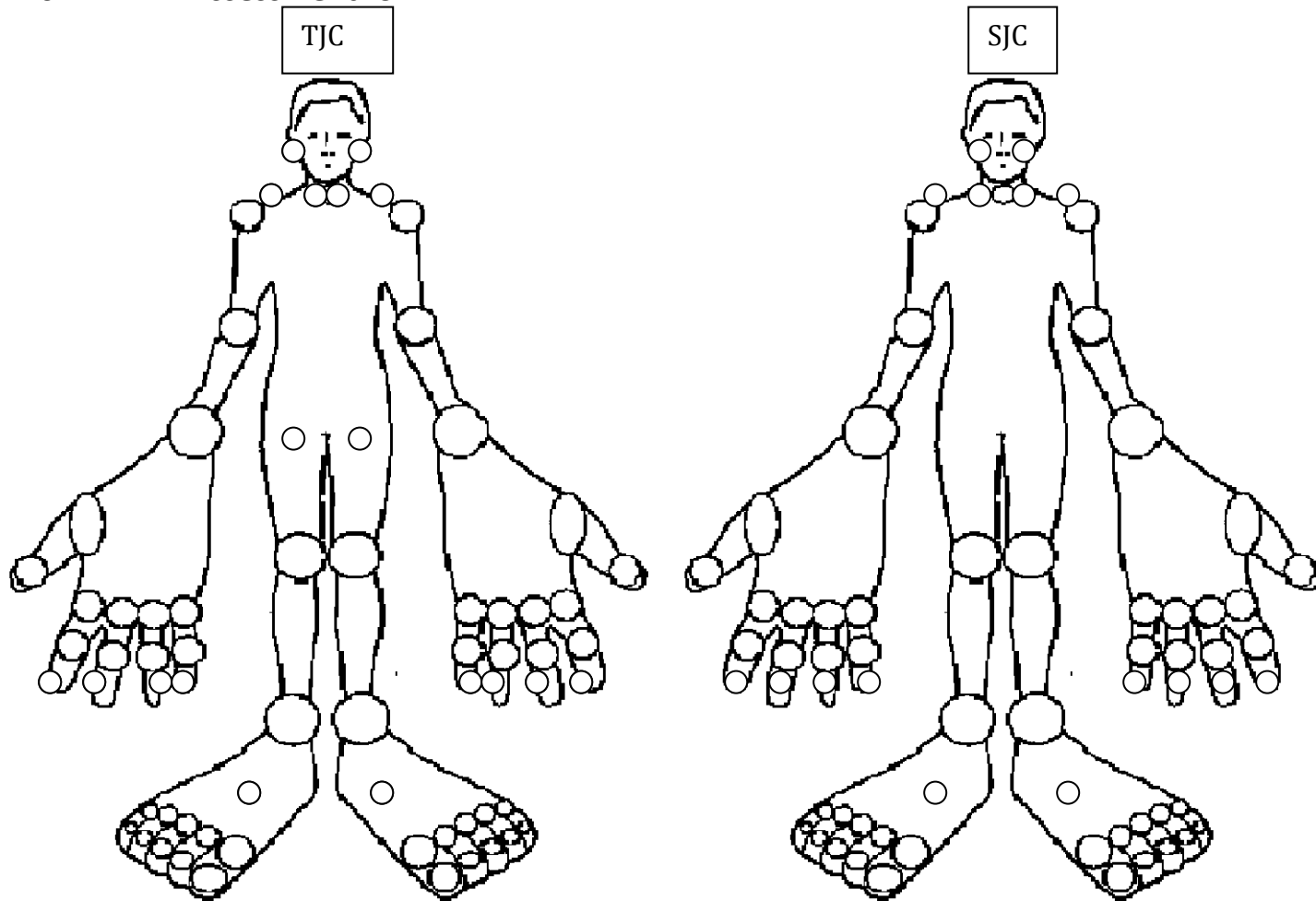
Pt Global(mm): Physician Global: Comment:
CRP: Fatigue: HSCRP:
ESR: Rheumatoid factor: AntiCCP:
TJ: EMS: Smoking: Nodular:
SJ: Modified HAQ: Sharpe:
DAS28: **Physician's Tender Joint Count** **Physician's Swollen Joint Count**
VASP:

28TJC: 28SJC:
66TJC: 66SJC:

EQ5D:
HAQ:
SF36:
IM Depo: ☐



11.6 REMIRA Assessment form



EMS:

Physician VAS:

Nodular?

Medication:

Comments:

Date:

11.7 Patient information leaflet for the REMIRA study (Healthy controls)

THE REMIRA STUDY - DEFINING LOW DISEASE ACTIVITY STATES IN RHEUMATOID ARTHRITIS USING CLINICAL, IMAGING AND BIOLOGICAL MEASURES

PRINCIPLE INVESTIGATORS: PROF DAVID SCOTT AND PROF ANDREW COPE

You are being invited to take part in this research project. Before you decide, it is important for you to understand why the research is important and what it will involve. Please take time to read the following information carefully. Talk to your family and friends about the study if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The treatment of Rheumatoid Arthritis has improved dramatically in recent years. The use of earlier and more intensive therapy means that the disease can now be controlled much more effectively. The aim of current treatment strategies is to induce a 'Low Disease Activity State' (LDAS), where there is minimal joint inflammation, which means minimal tender and swollen joints. Minimal joint inflammation is good as it reduces joint damage and disability. However, the way we currently measure disease activity in patients with RA in the clinic is not ideal for patients in LDAS. The aim of this study is to improve disease activity scoring systems for LDAS by using a combination of clinical, imaging (such as X-ray and ultrasound) and blood markers. This is important because some RA patients continue to develop joint damage in spite of low disease activity scores. This study will try to identify those patients who will develop further joint damage by measuring their white blood cells, the activity of genes that we think are involved in the disease process and by genetic testing. We hope in the future that we will be able to use the results of these blood tests to identify those patients with true LDAS in whom we can safely reduce therapy, as well as those patients likely to develop joint damage who would need to continue, or even, intensify therapy.

Why have I been invited to participate?

You are a healthy person with no evidence of rheumatoid arthritis. Your immune cells and genes function normally. We would like to compare immune cells and activity of genes in healthy individuals with RA patients. The differences and similarities will provide vital information of the different disease activity states in RA.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be asked to sign a consent form to show you have agreed to take part. You are still free to withdraw at any time and without giving a reason.

What will happen if I take part?

If you agree to take part, no more than 50ml of blood will be taken for research purposes. This will take about 10 minutes. You may experience slight discomfort and/or minor bruising as a result of giving the blood sample. If you wish to stop at any time during the donation, you are able to do so.

What are the possible benefits of taking part?

There will not be a direct benefit to you following your participation, but the information we get might help to develop new management approaches for patients with rheumatoid arthritis.

Will my taking part in this study be kept confidential?

Yes. We will follow ethical and legal practice and all the information about your participation in this study will be kept confidential. Once we have collected the blood, all identifiable tags will be replaced by a code. Only the researchers involved in the study will be able to view the identifiable data, which are kept in a secure place.

What happens to the results from this study?

Results from this study may be published in scientific articles or presentations, although you may not be made aware of these. You will not be identified in any reports of publications.

Will any genetic tests be done?

As part of this study, participants will have genetic testing done on blood samples to see if carrying different genes will influence whether people with arthritis achieve low disease activity states. Genetic tests for other, unrelated conditions will not be tested. Using a different technique, we will also compare the activity of a large number of genes using a technique called 'gene expression profiling'. This will measure the activity of thousands of genes at once to create a global picture of the function of cells in the blood. These tests may help us identify study participants whose disease is in remission and provide important clues as to why arthritis continues to cause joint damage in a subset of study subjects.

What if there is a problem?

If you have a concern about any aspect of this study, you should speak to the research team who will do their best to answer your question. If you remain unhappy and wish to complain formally, you can do this through the formal NHS complaints procedure. Details can be obtained from the hospital. In the unlikely event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against the NHS Trust administering your care. You may have to pay your legal costs. The normal NHS complaints mechanisms will still be available to you.

Who is organising and funding the research?

This project is funded by the National Institute of Health and Research (NIHR).

Who has reviewed the study?

All research in the NHS is reviewed by an independent group called a Research Ethics Committee to protect your safety, rights, well-being and dignity. This study has been reviewed by the Wandsworth Research Ethics Committee. Your hospital Research and Development Department has also approved this project.

We would like to thank you for considering taking part or taking time to read this sheet.

11.8 Patient information leaflet for the REMIRA study (Remission patients)

REMIRA STUDY: DEFINING LOW DISEASE ACTIVITY STATES (LDAS) IN RHEUMATOID ARTHRITIS USING CLINICAL, IMAGING AND BIOLOGICAL MEASURES.

Principle Investigators: Prof David Scott and Prof Andrew Cope

You are being invited to take part in this research project. Before you decide, it is important for you to understand why the research is important and what it will involve. Please take time to read the following information carefully. Talk to your family and friends about the study if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1 tells you about the purpose of this study and what will happen to you if you take part. Part 2 gives you more detailed information about the conduct of the study.

Part 1

What is the purpose of the study?

The treatment of Rheumatoid Arthritis has improved dramatically in recent years. The use of earlier and more intensive therapy means that the disease can now be controlled much more effectively. The aim of current treatment strategies is to induce a 'Low Disease Activity State' (LDAS), where there is minimal joint inflammation, which means minimal tender and swollen joints. Minimal joint inflammation is good as it reduces joint damage and disability. However, the way we currently measure disease activity in patients with RA in the clinic is not ideal for patients in LDAS. The aim of this study is to improve disease activity scoring systems for LDAS by using a combination of clinical, imaging (such as X-ray and ultrasound) and blood markers. This is important because some RA patients continue to develop joint damage in spite of low disease activity scores. This study will try to identify those patients who will develop further joint damage by measuring their white blood cells (T cells, B cells and monocytes), the activity of genes that we think are involved in the disease process and by genetic testing. We hope in the future that we will be able to use the results of these blood tests to identify those patients with true LDAS in whom we can safely reduce therapy, as well as those patients likely to develop joint damage who would need to continue, or even, intensify therapy.

Why have I been invited to participate?

Currently, the treatment you are receiving seems to be working very well and your disease is under good control. You fulfil the criteria for 'Low Disease Activity'.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be asked to sign a consent form to show you have agreed to participate. You are still free to

withdraw at any time and without giving a reason. A decision to withdraw at any time or a decision not to take part, will not affect the standard of care you receive.

What will happen if I take part?

You will be monitored in the outpatient's clinic for 1 year while you continue with your current treatment. You will be seen initially at the start of the study, and then three monthly until 12 months. The activity of your arthritis will be assessed in detail and you will be asked to complete questionnaires about the way in which the arthritis is affecting your life. We will also take blood tests during these visits. We will take no more than 50ml of blood (less than half a tea cup full) during each visit; these blood samples will be taken at the same time as the samples we take for routine monitoring of your treatment. Ultrasound scans of your joints will also be carried out some of these visits (similar to those used during pregnancy to visualise the foetus). At the beginning and end of the study, we will also carry out X-rays of your hands and feet. In total, these visits will last about 2 hours. These tests will help us to establish whether there has been any damage to your joints during this period of LDA.

What are the advantages of taking part?

You will be monitored more closely than routine care. In particular, the use of ultrasound and certain blood tests are not currently available to all patients in routine clinical practice. In the future, the aim will be to make these technologies available to all our patients if it is proven to be useful.

What are the possible disadvantages and risks of taking part?

Your arthritis treatment will not be altered because of this study, so there are no real disadvantages or risks from taking part in the trial.

Ionising Radiation (Medical Exposure) Regulations – IRMER

While all patients with a new diagnosis of Rheumatoid Arthritis will have X-rays taken of their hands and feet at the beginning of their treatment, it has become standard practice for patients to have X-rays carried out annually to monitor their treatment. Therefore, we will use these X-rays in this study to monitor progression of joint damage. The dose you will receive from the two sets of x-rays is very small and equivalent to 3 days of natural background level of radiation.

What happens when the research study stops?

All participants will continue with their routine clinical care.

Will my taking part in this study be kept confidential?

Yes. We will follow ethical and legal practice and all the information about your participation in this study will be kept confidential. The details are in Part 2.

Part 2

What if there is a problem?

If you have a concern about any aspect of this study, you should speak to the research team who will do their best to answer your question. If you remain unhappy and wish

to complain formally, you can do this through the formal NHS complaints procedure. Details can be obtained from the hospital. In the unlikely event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against the NHS Trust administering your care. You may have to pay your legal costs. The normal NHS complaints mechanisms will still be available to you.

Will my taking part in this study be kept confidential?

If you join the study, some parts of your medical records and the information collected for the study will be analysed by our team. The research team will have a duty of confidentiality to you as a study participant. We will do our best to meet this duty. All information that is collected about you during the course of the research will be kept strictly confidential, and any information about you that leaves the hospital will have your name and address (except your postcode) removed so that you cannot be recognised.

Involvement of your GP

We will notify your GP about your participation in the trial.

What will happen to any samples I give?

Only researchers and their designated collaborators involved in this study will have access to your blood samples. Upon collection, we will isolate blood serum, white blood cells, RNA and DNA from your blood using special techniques in the laboratory. These will then be analysed in the laboratory using a variety of different methods. We may need to store the samples for up to 10 years until we can perform a complete analysis. It is likely that new tests, not specified in this sheet, may become available for studying low disease activity. We therefore seek your permission to use your samples in future studies relating to disease activity. At the end of the 10 year period, all samples will be destroyed.

Will any genetic tests be done?

As part of this study, participants will have genetic testing done on blood samples to see if carrying different genes will influence whether people with arthritis achieve low disease activity states. Genetic tests for other, unrelated conditions will not be tested. Using a different technique, we will also compare the activity of a large number of genes using a technique called 'gene expression profiling'. This will measure the activity of thousands of genes at once to create a global picture of the function of cells in the blood. These tests may help us identify study participants whose disease is in remission and provide important clues as to why arthritis continues to cause joint damage in a subset of study subjects.

What will happen to the results of the research study?

The overall results of the trial will be collected by the Chief Investigators. We intend to present and publish the findings to inform others about this trial. This will take at least 4 years from the beginning of the trial. When the results are published, we will be happy to make them available to all those who took part. No individual study participant will be identified in any report or publication from this study. The results

will also be written up in detail and submitted to London University as part of a PhD degree.

Who is organising and funding the research?

This project is funded by the National Institute of Health and Research (NIHR) Doctoral Research Fellowship (DRF) awarded to Dr Margaret Ma.

Who has reviewed the study?

All research in the NHS is reviewed by an independent group called a Research Ethics Committee to protect your safety, rights, well-being and dignity. This study has been reviewed by the Wandsworth Research Ethics Committee. Your hospital Research and Development Department has also approved this project.

We would like to thank you for considering taking part or taking time to read this sheet.

11.9 Patient information leaflet for the REMIRA study (Active RA controls)

THE REMIRA STUDY - DEFINING LOW DISEASE ACTIVITY STATES IN RHEUMATOID ARTHRITIS USING CLINICAL, IMAGING AND BIOLOGICAL MEASURES

PRINCIPLE INVESTIGATORS: PROF DAVID SCOTT AND PROF ANDREW COPE

You are being invited to take part in this research project. Before you decide, it is important for you to understand why the research is important and what it will involve. Please take time to read the following information carefully. Talk to your family and friends about the study if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

The treatment of Rheumatoid Arthritis has improved dramatically in recent years. The use of earlier and more intensive therapy means that the disease can now be controlled much more effectively. The aim of current treatment strategies is to induce a 'Low Disease Activity State' (LDAS), where there is minimal joint inflammation, which means minimal tender and swollen joints. Minimal joint inflammation is good as it reduces joint damage and disability. However, the way we currently measure disease activity in patients with RA in the clinic is not ideal for patients in LDAS. The aim of this study is to improve disease activity scoring systems for LDAS by using a combination of clinical, imaging (such as X-ray and ultrasound) and blood markers. This is important because some RA patients continue to develop joint damage in spite of low disease activity scores. This study will try to identify those patients who will develop further joint damage by measuring their white blood cells, the activity of genes that we think are involved in the disease process and by genetic testing. We hope in the future that we will be able to use the results of these blood tests to identify those patients with true LDAS in whom we can safely reduce therapy, as well as those patients likely to develop joint damage who would need to continue, or even, intensify therapy.

Why have I been invited to participate?

You have rheumatoid arthritis and your disease is still active. Whilst your doctors are trying to reduce your disease activity, we would like to take a blood sample from you and to compare your immune cells and gene activity to someone with low disease activity. The differences and similarities will provide vital information of the different disease activity states in RA.

Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be asked to sign a consent form to show you have agreed to take part. You are still free to withdraw at any time and without giving a reason.

What will happen if I take part?

If you agree to take part, no more than 50ml of blood will be taken for research purposes. This will take about 10 minutes. You may experience slight discomfort and/or minor bruising as a result of giving the blood sample. If you wish to stop at any time during the donation, you are able to do so.

What are the possible benefits of taking part?

The information from the study may help to develop new management approaches for patients with rheumatoid arthritis. This will benefit you in the future when your disease is under better control.

Will my taking part in this study be kept confidential?

Yes. We will follow ethical and legal practice and all the information about your participation in this study will be kept confidential. Once we have collected the blood, all identifiable tags will be replaced by a code. Only the researchers involved in the study will be able to view the identifiable data, which are kept in a secure place.

What happens to the results from this study?

Results from this study may be published in scientific articles or presentations, although you may not be made aware of these. You will not be identified in any reports of publications.

Will any genetic tests be done?

As part of this study, participants will have genetic testing done on blood samples to see if carrying different genes will influence whether people with arthritis achieve low disease activity states. Genetic tests for other, unrelated conditions will not be tested. Using a different technique, we will also compare the activity of a large number of genes using a technique called 'gene expression profiling'. This will measure the activity of thousands of genes at once to create a global picture of the function of cells in the blood. These tests may help us identify study participants whose disease is in remission and provide important clues as to why arthritis continues to cause joint damage in a subset of study subjects.

What if there is a problem?

If you have a concern about any aspect of this study, you should speak to the research team who will do their best to answer your question. If you remain unhappy and wish to complain formally, you can do this through the formal NHS complaints procedure. Details can be obtained from the hospital. In the unlikely event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for a legal action for compensation against the NHS Trust administering your care. You may have to pay your legal costs. The normal NHS complaints mechanisms will still be available to you.

Who is organising and funding the research?

This project is funded by the National Institute of Health and Research (NIHR).

Who has reviewed the study?

All research in the NHS is reviewed by an independent group called a Research Ethics Committee to protect your safety, rights, well-being and dignity. This study has been reviewed by the Wandsworth Research Ethics Committee. Your hospital Research and Development Department has also approved this project.

We would like to thank you for considering taking part or taking time to read this sheet.

11.10 Consent Form - Remission Patients

Title of Project: TREATMENT DECISIONS IN RHEUMATOID ARTHRITIS: DEFINING LOW DISEASE ACTIVITY STATES USING CLINICAL, IMAGING AND BIOLOGICAL MEASURES.

Principle Investigators: Prof David Scott and Prof Andrew Cope

1. I confirm that I have read and understood the Patient Information Sheet dated 21/12/09 for the above study. I have had the opportunity to consider the information, ask questions and have these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I'm free to withdraw at any time without giving any reasons, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be used by the research team from King's College London and their designated collaborators, from regulatory authorities or from the NHS, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I understand that my blood samples will be used for purposes of laboratory research, including gene testing, by the research team and their designated collaborators. ☐
5. I consent for my samples to be used in future research studies. ☐
6. I agree to my GP being informed of my participation in the study. ☐
7. I agree to take part in the above study.

PATIENT

Name: _____

Signature: _____

Date: _____

PERSON TAKING CONSENT

Name: _____

Signature: _____

Date: _____

11.11 Consent Form – Healthy**Controls**

Title of Project: REMIRA STUDY - DEFINING LOW DISEASE ACTIVITY STATES
IN RHEUMATOID ARTHRITIS USING CLINICAL, IMAGING AND BIOLOGICAL MEASURES

Principle Investigators: Prof David Scott and Prof Andrew Cope

8. I confirm that I have read and understood the Patient Information Sheet dated 25/01/10 V1 for the above study. I have had the opportunity to consider the information, ask questions and have these answered satisfactorily. ☐
9. I understand that my participation is voluntary and that I'm free to withdraw at any time without giving any reasons, without my medical care or legal rights being affected. ☐
10. I understand that my blood samples will be used for purposes of laboratory research, including gene testing, by the research team and their designated collaborators. ☐
11. I consent for my samples to be used in future research studies. ☐
12. I agree to be contacted about participation in future studies ☐
13. I agree to take part in the above study. ☐

PATIENT

Name: _____

Signature: _____

Date: _____

PERSON TAKING CONSENT

Name: _____

Signature: _____

Date: _____

11.12 Consent Form - Active RA controls

Title of Project: REMIRA STUDY - DEFINING LOW DISEASE ACTIVITY STATES
IN RHEUMATOID ARTHRITIS USING CLINICAL, IMAGING AND BIOLOGICAL MEASURES

Principle Investigators: Prof David Scott and Prof Andrew Cope

14. I confirm that I have read and understood the Patient Information Sheet dated 25/01/10 V1 for the above study. I have had the opportunity to consider the information, ask questions and have these answered satisfactorily. ☐
15. I understand that my participation is voluntary and that I'm free to withdraw at any time without giving any reasons, without my medical care or legal rights being affected. ☐
16. I understand that my blood samples will be used for purposes of laboratory research, including gene testing, by the research team and their designated collaborators. ☐
17. I consent for my samples to be used in future research studies. ☐
18. I agree to be contacted about participation in future studies ☐
19. I agree to take part in the above study. ☐
-
-

PATIENT

Name: _____

Signature: _____

Date: _____

PERSON TAKING CONSENT

Name: _____

Signature: _____






Date: _____

Your Health and Well-Being






This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities. *Thank you for completing this survey!*

For each of the following questions, please mark an ☐ in the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
				
<input type="checkbox"/> ₁	<input type="checkbox"/> ₂	<input type="checkbox"/> ₃	<input type="checkbox"/> ₄	<input type="checkbox"/> ₅

2. Compared to one year ago, how would you rate your health in general now?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
				
<input type="checkbox"/> ₁	<input type="checkbox"/> ₂	<input type="checkbox"/> ₃	<input type="checkbox"/> ₄	<input type="checkbox"/> ₅

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

Yes, limited a lot	Yes, limited a little	No, not limited at all
▼	▼	▼

- a Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports ☐₁ ☐₂ ☐₃
- b Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf ☐₁ ☐₂ ☐₃
- c Lifting or carrying groceries ☐₁ ☐₂ ☐₃
- d Climbing several flights of stairs ☐₁ ☐₂ ☐₃
- e Climbing one flight of stairs ☐₁ ☐₂ ☐₃
- f Bending, kneeling, or stooping ☐₁ ☐₂ ☐₃
- g Walking more than a mile ☐₁ ☐₂ ☐₃
- h Walking several hundred yards ☐₁ ☐₂ ☐₃
- i Walking one hundred yards ☐₁ ☐₂ ☐₃
- j Bathing or dressing yourself ☐₁ ☐₂ ☐₃

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
▼	▼	▼	▼	▼

- a Cut down on the amount of time you spent on work or other activities ☐₁ ☐₂ ☐₃ ☐₄ ☐₅
- b Accomplished less than you would like ☐₁ ☐₂ ☐₃ ☐₄ ☐₅
- c Were limited in the kind of work or other activities ☐₁ ☐₂ ☐₃ ☐₄ ☐₅
- d Had difficulty performing the work or other activities (for example, it took extra effort) ☐₁ ☐₂ ☐₃ ☐₄ ☐₅

5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
▼	▼	▼	▼	▼

- a Cut down on the amount of time you spent on work or other activities ☐₁ ☐₂ ☐₃ ☐₄ ☐₅
- b Accomplished less than you would like ☐₁ ☐₂ ☐₃ ☐₄ ☐₅
- c Did work or other activities less carefully than usual ☐₁ ☐₂ ☐₃ ☐₄ ☐₅

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

Not at all	Slightly	Moderately	Quite a bit	Extremely
▼	▼	▼	▼	▼
<input type="checkbox"/> ₁	<input type="checkbox"/> ₂	<input type="checkbox"/> ₃	<input type="checkbox"/> ₄	<input type="checkbox"/> ₅

7. How much bodily pain have you had during the past 4 weeks?

None	Very mild	Mild	Moderate	Severe	Very Severe
▼	▼	▼	▼	▼	▼
<input type="checkbox"/> ₁	<input type="checkbox"/> ₂	<input type="checkbox"/> ₃	<input type="checkbox"/> ₄	<input type="checkbox"/> ₅	<input type="checkbox"/> ₆

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
▼	▼	▼	▼	▼
<input type="checkbox"/> ₁	<input type="checkbox"/> ₂	<input type="checkbox"/> ₃	<input type="checkbox"/> ₄	<input type="checkbox"/> ₅

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks...

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
	▼	▼	▼	▼	▼
a Did you feel full of life?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
b Have you been very nervous?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
c Have you felt so down in the dumps that nothing could cheer you up?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
d Have you felt calm and peaceful?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
e Did you have a lot of energy?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
f Have you felt downhearted and depressed?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
g Did you feel worn out?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
h Have you been happy?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5
i Did you feel tired?	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
▼	▼	▼	▼	▼
<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/> 4	<input type="checkbox"/> 5

11. How TRUE or FALSE is each of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
	▼	▼	▼	▼	▼
a I seem to get sick a little easier than other people	<input type="checkbox"/> ₁	<input type="checkbox"/> ₂	<input type="checkbox"/> ₃	<input type="checkbox"/> ₄	<input type="checkbox"/> ₅
b I am as healthy as anybody I know	<input type="checkbox"/> ₁	<input type="checkbox"/> ₂	<input type="checkbox"/> ₃	<input type="checkbox"/> ₄	<input type="checkbox"/> ₅
c I expect my health to get worse.....	<input type="checkbox"/> ₁	<input type="checkbox"/> ₂	<input type="checkbox"/> ₃	<input type="checkbox"/> ₄	<input type="checkbox"/> ₅
d My health is excellent	<input type="checkbox"/> ₁	<input type="checkbox"/> ₂	<input type="checkbox"/> ₃	<input type="checkbox"/> ₄	<input type="checkbox"/> ₅

THANK YOU FOR COMPLETING THESE QUESTIONS!

11.14 FACIT-F form

FACIT-Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. **By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.**

		Not at all	A little bit	Some- what	Quite a bit	Very much
III7	I feel fatigued.....	0	1	2	3	4
III12	I feel weak all over	0	1	2	3	4
Aa1	I feel listless ("washed out")	0	1	2	3	4
Aa2	I feel tired.....	0	1	2	3	4
Aa3	I have trouble <u>starting</u> things because I am tired.....	0	1	2	3	4
Aa4	I have trouble <u>finishing</u> things because I am tired.....	0	1	2	3	4
Aa5	I have energy.....	0	1	2	3	4
Aa7	I am able to do my usual activities	0	1	2	3	4
Aa8	I need to sleep during the day.....	0	1	2	3	4
An12	I am too tired to eat.....	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired	0	1	2	3	4

11.15 Health assessment questionnaire

We are interested in learning how your illness affects your ability to function in daily life. Please feel free to add any comments at the end of this form. Please tick one response which best describes your usual abilities over the past week

	Without Any difficulty	With Some Difficulty	With Much Difficulty	Unable To Do
1. DRESSING AND GROOMING				
Are you able to:				
a. Dress yourself, including tying shoelaces and doing buttons?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
b. Shampoo your hair?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
2. RISING				
Are you able to:				
a. Stand up from an armless straight chair?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
b. Get in and out bed?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
3. EATING				
Are you able to:				
a. Cut your Meat?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
b. Lift a full cup or glass to your mouth?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
c. Open a new carton of milk (or soap powder)	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
4. WALKING				
Are you able to:				
a. Walk outdoors on flat ground?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3
b. Climb up five steps?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3

Please tick any aids or devices that you usually use for any of these activities:

Cane (W) ☐ Walking frame (W) ☐ Built-up or special utensils (E) ☐
 Crutches (W) ☐ Wheelchair (W) ☐ Special or built-up chair (R) ☐
 Devices used for dressing (buttonhooks, zipper pull, shoe horn) ☐
 Other (specify).....

Please tick any categories for which you usually need help from another person:

Dressing and Grooming ☐ Eating ☐
 Rising ☐ Walking ☐

Please tick the one response which best describes your usual abilities over the past week

5. HYGIENE	Without any difficulty	With some difficulty	With much difficulty	Unable to do	SCORE	
Are you able to:						
a. Wash and dry your entire body?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3		
b. Take a bath?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/>	<input type="checkbox"/>
c. Get on and off the toilet?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3		
6. REACH						
Are you able to:						
a. Reach and get down a 5lb object (e.g. a bag of potatoes) from just above your head?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/>	<input type="checkbox"/>
b. Bend down to pick up clothing of the floor?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3		
7. GRIP						
Are you able to:						
a. Open car doors?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/>	<input type="checkbox"/>
b. Open jars which have been previously opened?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3		
c. Turn taps on and off?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3		
8. ACTIVITIES						
Are you able to:						
a. Run errands and shop?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3	<input type="checkbox"/>	<input type="checkbox"/>
b. Get in and out of a car?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3		
c. Do chores such as vacuuming Housework or light gardening?	<input type="checkbox"/> 0	<input type="checkbox"/> 1	<input type="checkbox"/> 2	<input type="checkbox"/> 3		

Please tick any aids or devices that you usually use for any of these activities:

Raised toilet seat (h) ☐ bath seat (h) ☐ bath rail (h) ☐
 Long handled appliances for reach (r) ☐ Jar opener (for jars previously opened) (g) ☐

Please tick any categories for which you usually need help from another Person:

Hygiene ☐ Gripping and opening things ☐
 Reach ☐ Errands and housework ☐

11.16 EuroQol/EQ-5D

By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

1. Mobility *(please tick **one** box)*

- ☐ I have no problems in walking about
- ☐ I have some problems walking about
- ☐ I am confined to bed

2. Self-care *(please tick **one** box)*

- ☐ I have no problems with self-care
- ☐ I have some problems washing or dressing myself
- ☐ I am unable to wash or dress myself

3. Usual activities e.g. work, study, housework, family or leisure activities *(please tick **one** box)*

- ☐ I have no problems with performing my usual activities
- ☐ I have some problems performing my usual activities
- ☐ I am unable to perform my usual activities

4. Pain/Discomfort *(please tick **one** box)*

- ☐ I have no pain or discomfort
- ☐ I have moderate pain or discomfort
- ☐ I have extreme pain or discomfort

5. Anxiety/Depression *(please tick **one** box)*

- ☐ I am not anxious or depressed
- ☐ I am moderately anxious or depressed
- ☐ I am extremely anxious or depressed

6. Compared with my general level of health over the past 12 months, my health state today is *(please tick **one** box)*

- ☐ Better
- ☐ Much the same
- ☐ Worse

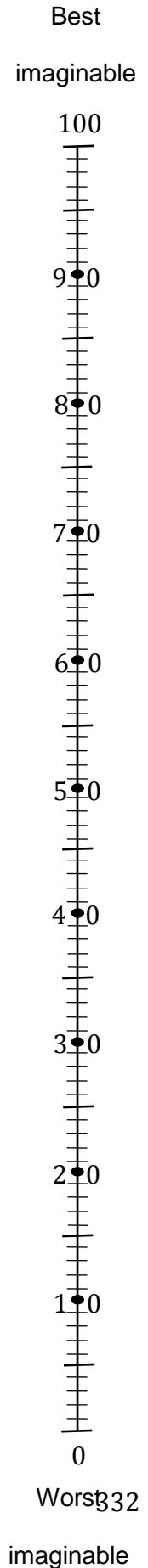
VISUAL ANALOGUE SCALE.

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

**Your own
health state**

Date



11.17 Summary of REMIRA study

